
nature

2 June 1977

Technology transfer at DOI

THE latest Report on Research and Development from Britain's Department of Industry (DOI), for the two years 1974–1976 (HMSO; £1.25), comes just at the time that Sir Ieuan Maddock hands over the post of Chief Scientist in the Department to Dr Duncan Davies, who was until recently in overall control of research at ICI. Research and development at DOI is not about to undergo sweeping changes as a result of this move, as the strategy for the next few years is bound to be evolutionary, but the report is valuable as marking more than just the end of one man's chieftainship—the Rothschild reorganisation of governmental support for science was also completed in the period under review, so it is possible to look at the whole new policy-making structure erected in the past few years.

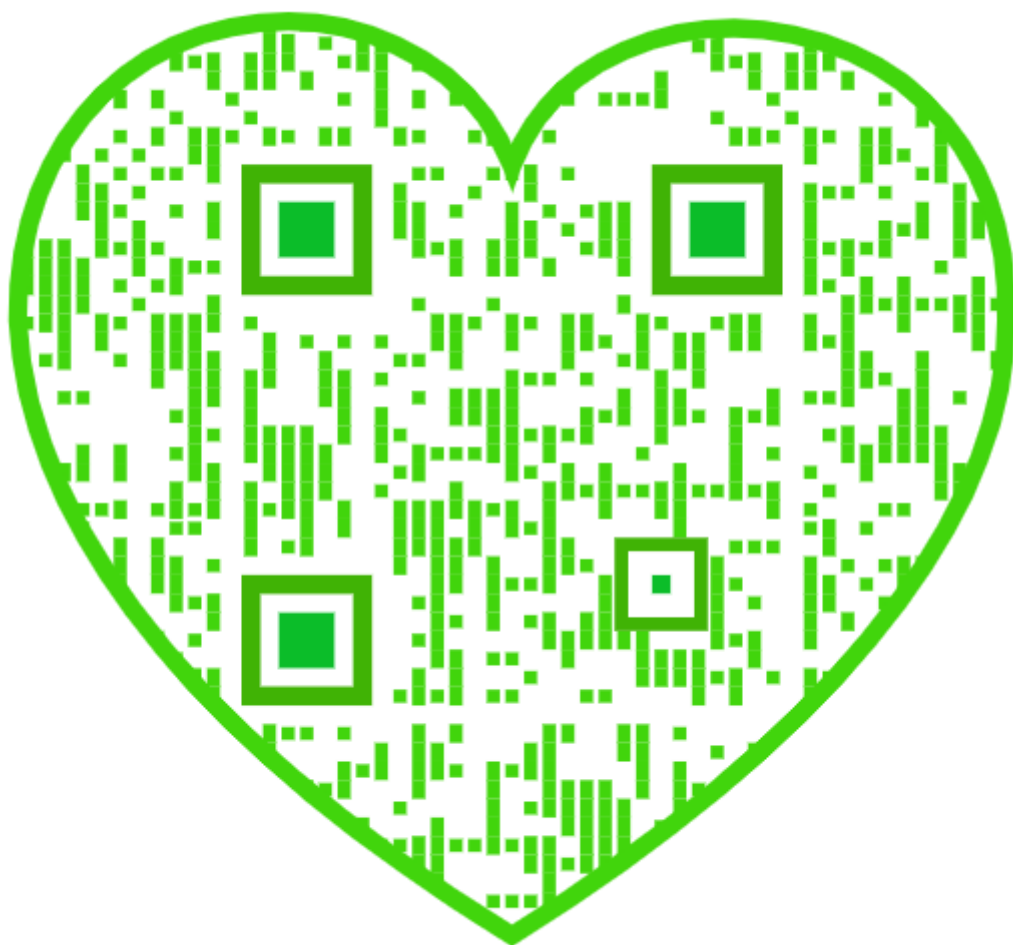
The department now spends roughly £100 million per year in industrial research and development, and of this about one-half goes into DOI research establishments (which include the National Physical and National Engineering Laboratories), and other governmental organisations. The remainder goes to industry, either directly (largely to the aerospace industry for the reduction of fuel consumption and noise) or indirectly by way of Britain's subscription to the European Space Agency of which DOI pays the major part. Away from the aerospace field, expenditure is widely scattered over subjects ranging from quantum metrology and waste recovery to cloth cutting for the garment industry. Research is guided by means of eight requirements boards, the membership of which is roughly half industrial, half academic and governmental.

The research and development aspect of the work is obvious and it is DOI's expressed intention to place more of its contracts out with industry itself, which should be welcome news for the not-exactly-buoyant industrial research sector. It should also place the governmental research establishments firmly on their

mettle to adopt aggressive programmes of their own, reaching out, as did Harwell several years ago with some success, in the hope of tapping new sources of funding. This will be a long haul. It is clear, however, that as the requirements boards begin to develop greater selectivity and acquire greater influence, they will want to avoid any pretensions that they act in some way as a sort of proxy management for the research establishments.

The other major aspect of DOI's task is to transfer technology out of the laboratory and into the factory. It has recently been a departmental theme that the national emphasis on aerospace has been to the detriment of the low-technology or small industry where the largely unmet demand is for modern rather than highly advanced technology. And it is in this field of the application of already existing technology that very interesting second careers are to be made.

We have repeated almost *ad nauseam* that the civil-servant scientist and technologist finds him or herself in an unfortunate bind in that seniority in the service leads, for most, not to more broad administrative and managerial responsibilities but to more of the same thing, often against a background of rapid and difficult-to-absorb change. In the field of technology transfer there could well be scope for the gainful employment of many who are at the present doing little more than marking time. For the skill that is most needed is not intellectual brilliance but wide experience. To be sure, not every one-time scientist or technologist is going to be able to develop into an ideas broker, capable of getting, in the most unabrasive way, into the textile mills or the workshops under the railway arches to hear about old problems and to talk about new techniques. But there must be some who would relish the challenge of such a job. Maybe DOI's new Chief Scientist will be tossing such an idea into the laboratories under his wing. □



Séveso sollicitude

Alastair Hay visits Séveso and finds political as well as technical problems

IT IS ten months since the ICMESA chemical plant reactor exploded discharging tetrachlorodibenzo-*p*-dioxin (dioxin) over a large section of the Italian town of Séveso. Though considerable progress in the reclamation of the largest residential portion of the area is now apparent, the record of success has not extended to actual physical destruction of the dioxin present in the contaminated material collected. Vacillation over the methods to be adopted is caused by several factors: a paucity of relevant scientific data, hostility by Séveso residents to some of the methods which the Lombardy regional government has proposed, and indecision in government circles at the regional and national level. At regional level the preferred course of action seems to be destruction by incineration, but Séveso residents view construction of the incinerator with alarm and this could prove electorally damaging to the political parties concerned.

Professor Augusto Giovanardi, coordinator of the Decontamination Commission appointed by the Lombardy regional government to supervise operations at Séveso, says that according to measurements made by the commission between 300 and 500 grams of dioxin were deposited in the most contaminated area (designated zone A) and that some 10 grams were present in the less affected zone B. He dismisses the suggestion made by a British scientist, Dr Donald Lee, that up to 130 kg of dioxin could have been produced in the reactor explosion. However, Giovanardi acknowledges that neither his commission nor Givaudan had been given permission by the Italian courts to check the reactor contents. This analysis was being carried out solely by scientists employed by the judiciary.

Seven sections

For the purpose of decontamination policy zone A has been divided into seven sections. Areas A1–A3—those nearest the reactor—are the most seriously contaminated, and contain about 80% of the discharged dioxin. A4 and A5 are areas of 'medium contamination'; A6 and A7, the least affected portions of zone A, housed 80% of the residents evacuated from the area by the local authority. Consequently it has been sections A6 and A7 which have received most attention by the authorities. Giovanardi explains

that buildings in the area have been decontaminated by a process of vacuum cleaning and washing with solvents. "Most of the soil and vegetation from the area had been stripped away two weeks ago", he says, "and the pavements and roads will be the last portions to be cleared". He adds: "We hope that the people with homes in the area will be able to return to them sometime in the months of June to August this year".

Concurrent with the decontamination work in zone A the commission has been working in zone B, which may yet have to be expanded. Here the residents have been asked to abide by certain rules to minimise personal exposure to dioxin. Children leave the area for the daylight hours, and women in the first trimester of pregnancy are advised to move from the district until the foetus is at least three months of age. With the work on sections A6 and A7 now nearing completion Giovanardi says the commission "is now applying the same operation to the houses, vegetable gardens and so on in zone B that we have used in A6 and A7". Contaminated soil and vegetation from these areas is being deposited in the 'medium contaminated' sections A4 and A5. The vegetation is to be incinerated, but the soil may well be left unprocessed in the hope that microbial degradation will reduce the dioxin concentrations to acceptable levels.

Whereas the consensus of scientific opinion is that the soil should be removed from zones A6 and A7 and definitely from the 'heavily contaminated' areas A1–A3, opinions differ on the methods to be applied to degrade the dioxin present. Incineration is apparently the approach favoured on theoretical grounds by some members of the commission, but this view is by no means universal. Other scientists are conducting trials on photochemical decomposition of dioxin, solvent extraction of the chemical (from the soil) and degradation by steam distillation.

The most vehement opposition to the incineration proposal and to the plans to construct an incinerator on the site of the ICMESA plant comes from the Séveso residents. They have had enough. If the incinerator has to be built, they argue that it should be built somewhere else. They know that incineration of the soil on the scale proposed will be an enormous under-

Sorry, for copyright reasons some images on this page may not be available online

Keystone

Barrier to progress?

taking. It will be an untried process and they claim that they are to be the 'guinea pigs' of the regional authority. In the past month they have mounted a demonstration in Milan to protest against the incinerator's construction and gunmen have broken into the office of the health officer at Séveso, shooting him several times in the legs.

Information relevant to the handling of dioxin-contaminated soil has been procured for the Italian authorities by Professor Barry Commoner from the US Department of Defense. On a visit to Italy in February and March, Commoner expressed concern and disappointment at the measures being adopted. He wrote that it was evident that "a systematic examination of alternatives [for degrading dioxin] has not been carried out" by the Decontamination Commission, and much of the decontamination work was "unnecessarily confused". Asked about Commoner's criticisms, Giovanardi's response is that whereas incineration appears to be the most feasible degradative process, his commission is considering all alternative procedures.

Government Commission

Last year the Italian Government appointed its own Commission to assist the local authorities at Séveso and the regional government in Lombardy. The Under Secretary of State for Health, Dr Fernando Russo, is the commission's president. As such he has the responsibility for collating all the available information on dioxin both nationally and internationally. He says there is "no evidence available on the complete destruction of dioxin. We are still searching for this evidence on an international scale".

Russo says that the final aim of all the authorities is to reduce the dioxin

levels to zero. The Ministry of Health convened a meeting in Rome recently to assess the situation at which a whole series of programmes were discussed. Many problems remained unsolved. Séveso has been a very bitter lesson for Italy. It has forced the government to review its legislation on industrial hazards, pollution control, toxicology and food additives, amongst other things. The message has been rammed home that 'prevention is better than cure'. Russo says Italy would like legislation on pollution control in particular to be a European Community measure, for national legislation on this issue "might provoke reprisals" and force multinational companies to go elsewhere.

As for the specific pollution problem at Séveso, Russo is in no doubt that ICMESA's owners Givaudan, as a big company, "will more or less have to pay" for the damage they caused, estimated now to cost 113 billion lira—about £80 million. Givaudan states that this figure seems "extremely large".

Just to ensure that they do receive payment, the local authorities in and around Séveso, the Lombardy regional government and the central government are all involved in litigation for damages against Givaudan. The company, they claim, had inadequate safety features operating in the trichlorophenol reactor. And to compound the problem, when the accident occurred the ICMESA management provided insufficient information on the pollution hazard. As a result many people were put to unnecessary risk by their exposure to dioxin for the two weeks before residents were evacuated.

Central matter

This two week period of exposure is central to the health problems of the Séveso population and to the issue of

abortion for the pregnant women exposed to dioxin. Although Russo claims that the Italian government wasn't against abortion for these women and that the opposition came from local doctors at Séveso, he has the impression that it hadn't been too difficult for the women to procure legal terminations.

This impression is not confirmed by Ms Adele Faccio, the Radical Party Member of Parliament for the constituency of Genoa. She was in the Séveso area in July and August last year and, as a result, has been particularly concerned about the question of abortions for the Séveso women.

"It was extremely difficult for these women to obtain legal terminations", she declares emphatically. To substantiate her claim she cites the figures for the abortions performed on the women. "Only 30 women had legal terminations", she says, "whereas twice this number have had illegal abortions. The illegal terminations were performed by 'women's self-help groups' for cases where the foetus was not older than ten weeks. For women in a more advanced state of pregnancy it was essential to send them abroad for an abortion".

But if legal abortions have proved difficult for the Séveso women, there is some relief in Italian medical circles over the results of the examinations performed on the foetuses from those women permitted legal terminations. These examinations were conducted by Professor Alfred Gropp and Dr Helga Rehder of the Lubeck Medical School, Germany, and Professor Francesco Céfis and Dr Laura Sanchioni of the Istituti Clinici di Perfezionamento in Milan. Of the 34 foetuses examined only one showed any signs of abnormal development. As with most of the foetuses examined, however, this one embryo was badly macerated—a common situation with induced abortions.

The results must therefore be interpreted with great caution. They could be suggestive in this one instance of foetal Down's syndrome. This, however, is a not uncommon foetal abnormality in most populations. Some UK clinicians think it is unlikely to be due to dioxin. Professor Gropp and his colleagues claim in a report to the Lombardy Regional Health Authority that "the results of the embryo-morphological studies do not give any hint for damage brought about by the action of exogenous agents (dioxin)". They add the rider, however, that the negative result must not necessarily mean that dioxin "does not bear a risk for the mother and foetus". The examinations had certain inherent limitations: minor malformations and particularly organ damage

are not detectable in the very young foetus; only a limited number of cases could be studied, and most of these embryos were incomplete and of different gestational age.

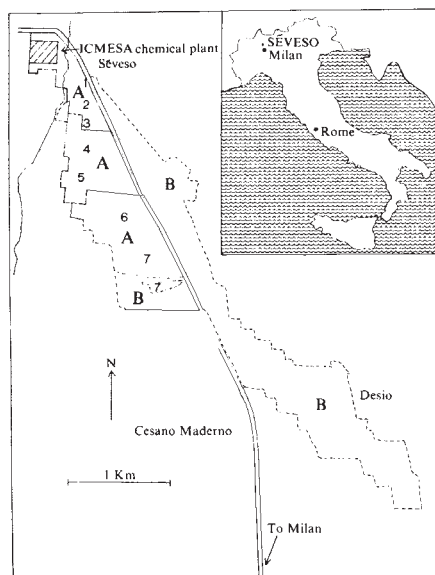
Professor Gaetano Fara, chairman of the Lombardy region's Health and Epidemiology Commission for Séveso, adds a further qualification to the interpretation of these results. He admits the foetal examinations cannot be interpreted in the context of a true epidemiological survey; they are an analysis of data to hand. He adds that, for a true epidemiological survey, the population would obviously have been much larger and more carefully monitored.

As for the eight children from the Séveso area born with abnormalities, Fara claims that statistics would indicate that this incidence of malformation is no greater than the average for the region. Some epidemiologists in the UK have expressed the opinion that because of the variation in the pattern malformations reported at Séveso it is quite possible that they are not due to dioxin. They add that, if the chemical was responsible for these abnormalities it would be reasonable to expect a more consistent pattern in the defects observed.

Even though some of the medical results appear to be reassuring, Fara insists that they still consider the Séveso residents to be a 'population at risk'. He said that a preliminary report on the children from Séveso based on three visits to doctors had revealed no immunological damage in this group, compared with their peers in a non-contaminated area. Similarly chromosomal studies performed on the 34 aborted foetuses by Dr Luigi de Carli at the University of Bazia had revealed no genetical deviations from the accepted norm.

But Fara does admit that the cases of chloracne reported represent an area greater than that officially designated as being contaminated. He acknowledges that it is worrying, but adds that of the 525 cases of reported chloracne, only 1 in 4 were confirmed on subsequent re-examination. In view of the toxicity of dioxin, however, the regional authority is to embark on a large scale epidemiological study of the area. Fara says that the population will have to be monitored for years to come.

If Professor Giovanardi's predictions are correct the Decontamination Commission will not complete its programme to bring dioxin down to an acceptable level before 1980. By this time most of the residents will have returned to their homes, but as a population still 'at risk' fears about dioxin will still be a part of their lives. □



Scientists and education in Poland

Social change in Poland is affecting the country's science and education policies. A **Special Correspondent** reports

DURING periods of heightened social tension, general issues dominate science and education policy, as the present situation in Poland illustrates. The main problems which face the country are political rather than economic. The beginning of a wave of repression against the participants in strikes in June 1976 (*Nature*, 5 August 1976) followed the abortive decision to raise prices significantly. On 23 September 1976 the Workers Defence Committee (KOR) was formed by a small group of courageous people. It made several demands; unconditional amnesty for all sentenced for their participation in strikes and demonstrations; provision of appropriate jobs for all workers dismissed from the factories and restoration of long-service privileges; disclosures of all repressions including beating by the police while in custody, false accusations and confessions coerced by force, unlawful conduct of courts and so on; and the setting up of an independent parliamentary committee to investigate all facts related to infringement of human rights.

Acting openly in spite of the fact that it is illegal, the KOR, consisting of 23 members, enjoys considerable support. It is collaborating with a fairly wide group, mainly students and recent graduates drawn from participants in the student riots of March 1968 and from the Clubs of Catholic Intelligentsia and, to a lesser degree, from the Scouting organisation. It is their devoted work that enables KOR to collect and distribute money (about 500,000 zlotys up to the end of March); to gain access to the people involved

and to monitor the court proceedings; and to publish and circulate KOR's *Bulletin* (nine issues so far) and other documents. KOR's appeal for public support has been answered by many letters of protest to the Polish Parliament, from more than 2,500 people. They include 730 students from the University of Warsaw, 520 from the University of Krakow, and 285 from the Catholic University in Lublin.

KOR is in no sense a political party: it has no political programme and the political opinions of its members and collaborators differ considerably. None of its activities is directly political, and this may be the source of its strength and early immunity. The tactics chosen by KOR have been effective; its very existence has structured internal political life and provided a new pattern of defence of civic rights under the communist regime. This is manifested in the growing self-assertion of diverse social groups in exercising their rights under existing laws. Writers have produced a first unofficial literary journal *Zapis*, a large type-written volume of poetry, stories and criticism. A number of political programmes and groups have appeared, some of them critical of KOR.

Scientists have also been active. Thirty-four professors, most of whom have not raised their voice in public before, sent a letter of protest to Parliament. They included many well-known scientists and members of the Polish Academy of Sciences (PAS): W. Kemula (Chemistry), W. Miemierko, J. Kielanowski, W. Kunicki-Goldfinger, E. Domanski, A. Brzoza, M. Nunberg

(Natural Sciences), I. Chmielewska (Biochemistry). Another letter was signed by about 300 younger scientists and artists. Although these letters are the most visible protestations, others are no less significant. During the General Assembly of the PAS on 17 December 1976, for example, several members of the Academy opposed the established procedure which reduces the role of the Assembly to a purely formal acceptance of candidates proposed by the Praesidium (which in turn is completely dependent on the political authorities). Three recommended candidates were not elected. Among them were Professor J. Szuba (favoured personally by Mr Gierek, the Secretary of the Party) and Professor Z. Rybicki, the Chancellor of Warsaw University.

Teachers' protest

Some letters of protest were signed by school teachers. In Poland the teaching profession is particularly weak because for many years it absorbed weaker graduates unable to find jobs of higher social standing and independence from administrative bodies; also the teaching profession consists almost entirely of women. The teachers' publicly expressed protests were followed immediately by repressions. In the Tadeusz Rejtan secondary school in Warsaw, for example, six teachers were given dismissal notices (three of them are to be transferred to other schools, and three have been asked to retire early; these decisions have not yet been implemented). Ironically Tadeusz Rejtan was the member of Parliament who fought against the first partition of Poland in 1773. The action against teachers immediately provoked a new flow of protests, this time from the school's students and graduates.

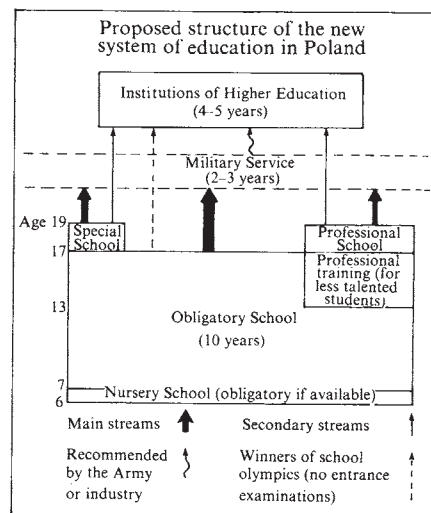
No similar case of repression of older members of the scientific community is known to this correspondent,

Miloslav Chojceki, a nuclear physicist, and Piotr Naimski, a biochemist, were recently arrested as part of a new campaign of the Polish Government against the Workers' Defence Committee. In all, some 25 activists were arrested, apparently as a preventive measure. The recent death, in mysterious circumstances, of Krakow student Stanislaw Pyjas has led to renewed agitation against alleged police illegalities, and it is thought that the arrest of the activists was intended to neutralise this opposition. In spite of the arrests, however, the agitation, and demands for a public inquiry into the death of Pyjas, are still increasing.

Camera Press

Sorry, for copyright reasons some images on this page may not be available online

Warsaw University library



and the authorities try not to create martyrs. The situation for younger scientists, however, is different. The case of Mr Chojecki, who was dismissed from the Institute of Nuclear Research, has already been mentioned in *Nature*. More recently Mr J. Celinski, who was dismissed from the University of Warsaw, brought his case to court. The court upheld the decision of the university because, as formally stated in the judge's justification,

Each department and its chief is responsible for the results of work and is free to choose employees with appropriate professional qualifications. These qualifications include sometimes . . . political criteria . . . and the political convictions of the plaintiff disqualify him from being a member of staff of the University of Warsaw.

These actions against teachers and scientists are of course relatively mild. Under present international circumstances, however, it is precisely the activity of KOR that makes the escalation of repression difficult. Each country has its own scale of values and measures of tolerance which form an ethical frame in which the political repressions in Poland should be judged.

Another major determinant of the situation is the plan for a new educational system. Parliament passed a curious bill describing the general shape of future changes but without specific decisions. The ten-year school, the basis of the new system (see figure), is not a secondary school in the ordinary sense, because entry to the university is guaranteed only for the winner of school olympics (a special type of competitive examination) and for those who have a record of "two years of excellent professional work or two years of impeccable military service".

The most striking feature of the new system is that most future students will have to complete military service before entering university. However, there will be other options for students leaving the ten-year school, ranging from six-month professional courses to two years of further general education. One can expect therefore a highly differentiated level of readiness for university studies which must impinge on the performance of the universities. The further feminisation of the universities would also follow. The fraction of women in institutions of higher education is about 52%; arts faculties have 77%; education 74%; medicine, with some measure of preference for men, has 64%; engineering, the least feminised faculty, has 34%.

Implementing the plan

Preliminary steps for introduction of the new educational system have

already been taken. About 70,000 acting teachers are being taught in special schools or special faculties of universities. It is commonly accepted that the courses they offer are poor, but they are entitled to confer masters degrees.

No one can deny that the present schooling system has serious shortcomings and does not serve the social needs of Poland. However, it is also true that discussion of the new system was deliberately channelled on to such problems as curricula, teacher training, availability of buildings and so on, and was made to bypass the evident political motives underlying the reform. The first of these is the failure of the schools' ideological influence; both teachers and students have adopted a sort of newspeak understood by both sides to be meaningless. Second, the schools do not provide appropriate political filters; in spite of some incentives, the number of students from workers' and farmers' families is not increasing and these students are in no sense more loyal to the party than other students. The authorities hope that the new system will provide more effective social indoctrination and more reliable political filters.

It is still unclear when implementation of the new system (planned for 1978) will begin. The system is very costly and its social price—the loss of experience accumulated by the existing system—is even higher. Postwar social changes in Poland opened the possibility of study for all who desired it, so making possible the social promotion of all gifted people from the working class. The byproduct of this process, however, was a significant decrease of the working class's own intelligentsia, a group which contributed most to the 'striving for education' within its class. Those gaining education often break their ties with their previous social environment (formally too they are not considered as members of the working class any longer) and constitute a very conservative group of people who, as they see it, have nothing to win and everything to lose.

There are additional reasons for the declining attractiveness of university studies for young people from the working class. Nowadays students who cannot rely on their families for support are in a worse position than their colleagues in the 1950s, because of a decrease in the real value of scholarships. More importantly, graduate prospects are not very bright: it is not easy to find appropriate jobs, salaries are relatively low, and there are serious difficulties in getting a flat (formally graduates have a high priority in the queue for flats, but the overall short-

Sorry, for copyright reasons some images on this page may not be available online

Camera Press

The Palace of Culture, seat of PAS

age makes this privilege of negligible value). Usually the new graduate is worse off than his less gifted friend from the primary school.

The political control of universities is exercised primarily by the careful selection of staff. No one can be engaged without the consent of the party organisation and without a good record from the police. The admission to postgraduate studies is similarly controlled, and several people who pass the examination are not admitted because of their or even their parents' political attitudes. In the recent enactment of the Ministry of Science and Higher Education (December 1976) it is openly stated that to be admitted to postgraduate studies the candidate has to "display proper ideological and civic attitudes". The chosen faculty has little relevance in this regard and candidates in social sciences and, say, medicine are treated on the same basis.

The fate of the new movements which are changing considerably the pattern of internal politics in Poland and, more generally, the immediate future of this country, are closely related to the whole human rights issue. This makes the proceedings and the results of the Belgrade Conference vitally important for Poland. At best one can expect some softening of the political line, and a more elastic and realistic approach to the needs and demands of people which could lead to a better economic performance. At worst one can foresee a partial return to cold-war policy with the rise of police power and a further widening of the gap between the rulers and the ruled. But even in this case the experience gained during the last few months could play an important role: Polish society has learned a lesson which it will be difficult to erase. □

USA

The threat to academic science

Colin Norman reports from Washington on a study of the state of US academic research

ACADEMIC scientists in the United States have been complaining bitterly during the past few years. Declining budgets and increasing amounts of red tape are putting severe strains on the country's research system and gradually eroding the quality of basic research, they claim. Yet American science remains outstanding by any measure: total funds for research and development are expected to reach about \$41,000 million this year, and scientists from the United States walked off with every Nobel Prize awarded last year. So do the complaints have any real basis, or are they simply self-interested pleas for more money?

According to an impressively detailed and closely argued report published this week*, there is indeed plenty of cause for concern about the health of academic research in the United States. Written by two social scientists working with a grant from the National Science Foundation (NSF), the report reaches this ominous conclusion: "The discoveries that are now being made are based on research initiated many years ago, the system is operating on momentum, but there are disturbing signs that the momentum generated during World War II and for 25 years thereafter has been halted. An important national resource may be eroding and the nation is faced with the possibility that it will lose scientific and technological leadership in many fields".

The authors of the study, Bruce L. R. Smith of Columbia University and Joseph R. Karlesky of Franklin and Marshall College in Pennsylvania, base that lugubrious assessment on a number of factors which are hurting the universities in general and university science in particular. Their chief message is not that academic science is in imminent danger of collapse, but that a number of far-reaching changes are taking place in the research system. "Failure to deal adequately with the long-term adverse trends could generate a decline from which recovery will be difficult and protracted", they warn.

In particular, Smith and Karlesky suggest that the problems afflicting university research could lead to

greater stratification in the higher education system, with fewer universities conducting research of the highest quality. There could also be a weakening of the traditionally close links between research and teaching, and funding constraints could lead to less support for innovative and speculative research, they warn. But the potential changes are not all bad, for Smith and Karlesky note that in 1967 the between universities and industry, after a considerable period of arms-length relationships has begun to take root again".

The most obvious problem affecting academic research is that federal support for research and development abruptly shifted gear in the late 1960s, moving from a period of burgeoning growth to a period in which total funding failed to keep pace with inflation. Smith and Karlesky cite figures, for example, which indicate that federal expenditures on basic research dropped by about 15% in constant dollars between 1968 and 1976. And that abrupt shift coincided with a turn for the worse in the general financial health of the universities as many sources of public and private funds began to dry up while overhead costs began to increase by leaps and bounds.

Superimposed on that trend of shrinking total support has been a marked change in the nature of federal funding for academic science. Smith and Karlesky note that in 1967, the federal government spent \$1,301 million to support specific research projects in colleges and universities, but another \$1,022 million was provided in the form of general research support (block grants to institutions, graduate traineeships and grants for improving general scientific capabilities). But by 1975, that pattern had changed dramatically: support for specific projects had increased to \$2,222 million while general research support had dropped to only \$567 million. "This substantial decline in institutional funding means that universities have at their disposal less discretionary money to fill gaps, start new exploratory studies . . . and meet other special and changing needs". In short, universities have lost considerable flexibility in the use of federal research funds.

A major impact of the decline in financial support has been a marked deterioration in the quality of scientific instruments and other laboratory equipment in the universities, as "a number of universities have postponed the replacement of equipment and deferred maintenance", Smith and Karlesky

suggest. They cite, for example, the case of one public university in the midwest which has been unable to provide any funds for scientific instruments for the past six years, and they suggest that the problem of replacing outdated instruments and poor facilities is now facing even the most prestigious universities.

It should be noted, however, that the NSF budget for fiscal year 1978, proposed by President Ford shortly before he left office, includes some \$38 million for upgrading instruments in university laboratories. The NSF budget is, however, encountering some difficulties in Congress, and it is by no means certain that even that small relief measure will be fully approved.

A second major factor affecting the health of university research has been a marked deterioration in the academic job market for young scientists, as university expansion abruptly ceased in the early 1970s and the proportion of faculty members with tenure has increased sharply. Though there are significant differences between universities and between different fields of science, Smith and Karlesky report that the proportion of faculty members occupying tenured positions increased from 47% in 1969 to 65% in 1973. Moreover, according to a survey conducted by NSF, the tenure ratio in 1974 varied from a low of 59.1% in physiology to a high of 80.7% in chemical engineering.

"There is a growing uneasiness that the career pipelines may be too restricted and that determinants other than excellence are increasingly controlling career patterns", Smith and Karlesky report. They suggest, moreover, that "the decline in research opportunities for young investigators will be one of the most serious threats to the momentum of university research over the next decade". One noteworthy impact of the tenure logjam is, in fact, already showing up: the proportion of young faculty members (those who have held a PhD for less than seven years) declined from 43% in 1968 to 27% in 1975, and a further decline is anticipated by 1980.

As for enrolments in graduate departments, there was a major drop in the physical sciences and engineering in the early 1970s, while enrolments in the life sciences and the social sciences continued to increase. In some institutions, Smith and Karlesky predict that the rapidly shrinking numbers of graduate students could lead to decisions to drop some PhD programmes, a move which would force a painful choice between continuing to mount a research effort in the absence of graduate students or reducing re-

**The State of Academic Science* (Change Magazine Press, New Rochelle, New York; \$5.95).

search efforts to concentrate on teaching undergraduates. Such a decoupling of research and teaching functions would represent a significant departure from the traditional university role, but Smith and Karlesky note that some university departments are already contemplating whether to continue as research centres.

One of the major findings of the study was that recent trends in funding and enrolments have been felt most keenly by less distinguished departments and by less distinguished investigators. "In contrast", Smith and Karlesky report, "the leading science departments have tended to retain their relative strength . . . the downward trend has not appeared uniformly throughout all second-rank institutions, and the indicators of trouble have not always reached an advanced state. But the signs are sufficiently clear to warrant the conclusion that a very rapid deterioration in the relative position of many weaker departments could well occur in the near future".

In a sense, Smith and Karlesky note, it is "highly reassuring that our system of awarding research grants and contracts according to scientific merit appears to have achieved its aim in making certain that pre-eminent investigators are able to continue their work, and in most cases with adequate support". Nevertheless, the increased stratification in university research departments and the possibility that some second-rank institutions will drop research all together, raises a number of fundamental questions.

Although PhD programmes probably over expanded during the 1960s and "some of the research undertaken a decade ago was of less than first-rank quality", how far should the drift toward fewer and fewer outstanding research departments be allowed to proceed? Smith and Karlesky argue that "it is possible that the competitive elements in the system could be undermined if there were too few universities fully equipped for pre-eminent research. The nation would then be forced to rely on a narrow base of dominant centers".

In any case they argue that the trend "towards fewer universities and departments able to mount first-class research efforts will continue so long as federal research support remains essentially level, which is likely to be the case to some extent. Universities may be able to preserve their research missions by achieving a functional division of labor among themselves and by developing highly specialised research programs. But there is a certain minimum coverage of basic science fields that a university must undertake to retain its research vitality". □

200-mile zones: the threat to marine science

AS THE sixth session of the gruelling Law of the Sea Conference opened in New York last week, the National Academy of Sciences warned that the latest draft treaty could "cripple" marine scientific research. The Academy's warning, contained in a letter sent to Elliot Richardson, head of the US delegation to the conference, claimed that oceanographic research is in fact already being seriously hampered by restrictions imposed by some coastal nations.

The chief concern is that the draft treaty now under discussion (called the Revised Single Negotiating Text, in United Nations parlance) would give coastal states the right to regulate scientific research conducted within 200 miles of their shores. Since the coastal regions contain some of the most interesting oceanographic phenomenon, such as the major currents, virtually all of the oceans' biological activity and most of the world's undersea earthquakes, the Academy is worried that the treaty could put major areas of scientific research off-limits.

The revised single negotiating text would require that the consent of a coastal state be obtained before a research project is conducted within 200 miles of its shore. Consent could be denied for research which "bears substantially on the exploration of the living and non-living resources", involves drilling, or interferes with fishing or other economic activities. In addition, the coastal state would have the power to veto publication of research results from some types of projects, and it would have the right to halt a project if it believed that the research differed from the description given in the original proposal. Such restrictions could "cripple future marine scientific research which will be critical to the survival of the oceans and mankind", the Academy stated.

The Academy is therefore urging that the draft be modified to provide the following conditions for research projects conducted outside a nation's territorial water (which will probably be 12 miles), but inside the 200-mile 'Economic Zone':

- Freedom of research should be guaranteed except for carefully specified and limited types of projects.
- Specific criteria should determine whether or not a project requires prior

consent, and a definite procedure should be established for obtaining consent. The chief objective would be to ensure that no unreasonable or arbitrary restrictions are imposed.

- Freedom to publish and disseminate research results should be preserved.

In addition, the Academy recommends that the nation undertaking the research should be required to keep the coastal state fully informed of the nature, objective and schedule of a proposed project. The coastal state should also have a right to be represented in the project, and it should be given preliminary and final reports, a share in the data and samples, and assistance in interpreting the results.

To support its contention that the provisions contained in the draft treaty would seriously hamper research, the Academy cites evidence that a number of coastal states are already exercising strict controls over projects within 200 miles of their shores. In particular the Academy notes that "in the past year the records of the US National University Oceanic Laboratory System, which coordinates the activities of the academic fleet, indicate that about half of the scheduled cruises for work in waters over which other nations claim control have been cancelled because requests were denied, or have been hindered sufficiently to prevent the cruise taking place. Some requests were never acknowledged; sometimes approval came too late for the program to be successfully conducted. At least 18 nations were involved in one way or another in inhibiting science in this way".

In spite of the Academy's strong plea, oceanographers do not hold much hope that the final version of the Law of the Sea Treaty (if such a document ever emerges) will protect marine research from arbitrary or unreasonable restrictions. Coastal states sometimes claim that research vessels are used as a cover for military or commercial operations and their activities should therefore be tightly monitored and controlled. Moreover, since the oceanographic research fleet belongs almost exclusively to the developed countries, there's little immediate incentive for developing countries to grant freedom of research within their economic zones.

Colin Norman

USSR

● The gap between R&D and the implementation of results is a common complaint against the whole spectrum of Soviet industry. In most cases the problem is simply one of logistics, the reorganisation of production plans and the re-equipping of plants without interrupting quarterly production schedules. For high-vacuum technology, however, the problem is more complex, as there is no single body responsible for R&D in this field. According to a recent *Pravda* article, the Ministry of Chemical and Petroleum Equipment, is prepared to tolerate a "considerable" gap between production and requirements during the current five-year plan; the Ministry of the Communications Industry, which needs the results of research in this field, will not itself undertake it "considering it to be the business of other branches."

No single body or ministry, it would seem, has high-vacuum technology high on its list of priorities. It is the Cinderella of research subjects, and it is not surprising, therefore, that a report on a pilot cryogenic 1,000 kW electric generator at present under test in Leningrad speaks gloomily of the technical problems involved, although it suggests that plans for a similar generator of up to 2 GW are already in operation.

Last December, an article by G. Saksaganskii, Head of the All-Union Section of Vacuum Apparatus, suggested a way out of this problem: the establishment of a 'Scientific-Technological Union' which would be responsible for satisfying all requirements in this field, over-riding ministerial and departmental barriers. He put particular emphasis on the need for custom-built apparatus for highly specialised branches of research and industry, and praised the high levels attained in this field by foreign firms.

A recent edition of *Pravda* says that Saksaganskii's article provoked a wide response from readers in industries which have some concern with vacuum technology. All the letter-writers "unanimously" supported his proposal for a single organisation to deal with "specialised" production. But, it is claimed, the letter-writers went even further: not only does industry require a special body to ensure production, there should also be a unified authority in charge of R&D. A number of writers proposed that a special scientific council should be established in the Academy of Sciences of the USSR "to coordinate theoretical research in the field of vacuum technology and the practical implementation of their results". In

addition, steps should be taken to ensure that there are sufficient number of highly-qualified personnel in the field of vacuum technology. So far, it is said, the ministries whose sphere of competence touches upon high-vacuum technology have made no comment on these suggestions.

A press campaign of this type is a traditional precursor of a major change in Soviet policy, and the response to Saksaganskii's article seems



to reflect a genuine discontent. The Soviet press frequently carries eulogies of the benefits of centralised planning. The current state of the high-vacuum industry, is, however, a salutary warning of what may occur when a particular branch or field of development is somehow omitted or overlooked, if not in the plan itself then in the practical details of implementation.

● The Byelorussian SSR is traditionally one of the poorest areas in natural resources of the whole of the Soviet Union. When, some 20 years ago, a large oil-refinery was built at Nova-Polatsk, the site was presumably chosen for geographical reasons, being close (in Soviet terms) to the Comecon countries which would be provided by pipeline with Soviet oil. Since then, however, oil has been located in the Byelorussian Republic itself, giving a yield in 1976 of some millions of tonnes. Some seventeen oil deposits have been located to date, and new techniques have been developed permitting the exploitation even of those around Mazyr, where the geological structure is not amenable to standard drilling techniques.

The search for oil has also yielded serendipitous results: potassium and rock salts, lignite, mineral resources, building stone and also non-ferrous metals have been discovered, as well

as new sources of drinking and spa waters. This mineral wealth has come to light in an area of Irish-type rural economy where even collectivisation could not break the traditional dependence on potatoes, peat, and cattle-rearing. The development and exploitation of the new resources should provide a fascinating study in years to come.

● In May 1976, *Pravda* proclaimed that the ninth five-year plan (1971-76) had been a period of intense implementation of laser technology in the national economy, stating that every rouble invested in laser R&D has yielded some 4-5 roubles of income. A number of practical applications of lasers were noted, such as the hardening of dies and metal-working instruments and the drilling of jewels for watches; more significant however, was the fact that an actual investment to expenditure ratio was mentioned, a comparative rarity in Soviet reportage which presumably indicates a particularly favourable return.

Since the current five-year plan began, the media have described a number of more exotic uses of lasers. These range from acupuncture (a recognised medical technique in the USSR) to the use of a laser-beam guidance system for the blind landing of aircraft at night or in adverse weather conditions. At the Latvian State University's astronomical observatory, a senior scientific worker, Maris Abele, and a senior engineer, Augusts Rubans, recently received the Silver and Bronze medals respectively of the Exhibition of All-Union National Economic Achievements for developing a laser range-finder for tracking satellites and space-probes.

Laser irradiation has been used to encourage the germination of seeds, and the Central Aerological Industry of the Hydrometeorological Service has produced a special laser reflector which is now being used for tsunami research in the Kuriles. Laser direction finders for determining the direction of mine-galleries are in use and are being exported to other members of the Comecon bloc. Naturally, nothing is said in these enthusiastic reports about possible military applications of the laser, and the outsider is left free to judge whether this emphasis of the peaceful and diverse use of lasers covers a classified programme, or whether, as in the now-notorious case of titanium alloys, the prolific references in the open press are evidence that no breakthrough of military significance has yet occurred.

Vera Rich

BRITAIN

Advice and consent

The UK government last week published a White Paper (Cmd. 6820) in response to the Flowers Commission report on nuclear power and the environment. Chris Sherwell reports

THE bare bones of the UK government's 17-page response to the Flowers report give those involved in Britain's nuclear debate something to chew over, but many will wonder whether it was worth the eight-month wait. The White Paper says the government has decided to accept the bulk of the Flowers Commission's recommendations. The most concrete action proposed concerns management of radioactive waste. The government also plans to broaden the range of advice it receives, and wants more information made available to the public to facilitate full discussion.

It is only at the end of the White Paper and in the context of public discussion that one of the hard foci of Britain's nuclear debate, the demonstration commercial fast breeder reactor (CFR1), actually surfaces. The government accepts, says the White Paper, that before any decision is taken on CFR1, a special procedure for achieving a proper framework for wider public debate should be settled and announced. Presenting the White Paper last week, the Environment Secretary, Mr Peter Shore, confirmed that CFR1 would not proceed until there had been a broad-ranging examination and debate. He cited the machinery of the Public Inquiry Commission which was available but had not been used as one possible course.

In accordance with the Flowers Commission's recommendation responsibility for the management of radioactive waste has now been given to the Environment Secretary together with the heads of the Scottish and Welsh offices. The government has also accepted in principle Flowers' suggestion for a Nuclear Waste Management Advisory Committee, though it thinks that initially this need not be a statutory body. It also sees no need for a decision yet on the suggestion for a Nuclear Waste Disposal Corporation, but says it will reconsider this in light of a review of existing arrangements for the control of waste which the government is now, according to the White Paper, carrying out "as a priority task".

On both waste management and radiation standards the White Paper announces important changes affecting the control of research. The Secretaries

of State for the Environment, Scotland and Wales will "assume control of the waste management element" in the UKAEA's total R&D expenditure. And responsibility for initiating and co-ordinating research into the effects of radioactivity on man and the environment can, says the White Paper, "be more appropriately performed by a Minister than (as the Commission suggested) by the National Radiological Protection Board (NRPB)".

Mr Shore amplified on this reasoning last week only to say both research and policy were in the public domain. Responsibility would again be exercised by the three ministers acting jointly. So will "strategic responsibility" for monitoring environmental pathways lie with the ministers rather than with the NRPB. The government does accept the Commission's recommendation that the NRPB should have a statutory responsibility for advice on the adequacy of radiation standards for workers and the general public. The membership of the NRPB and its sources of finance are now being reviewed.

On nuclear safety generally the advice the government now receives from the Nuclear Installations Inspectorate, which is part of the Health and Safety Executive, will be supplemented by advice from another independent and expert source. This will be the Advisory

Committee on the Safety of Nuclear Installations, which is being set up in place of the former Nuclear Safety Advisory Committee to advise the Health and Safety Commission.

The pieces of this institutional jigsaw will be increased further by the creation ("pretty soon", according to Mr Shore) of what the White Paper calls a high-level independent body "to advise specifically on the interaction between energy policy and the environment". And there is still Mr Benn's Energy Commission to come.

The White Paper emphasises that a decision both on the choice of thermal reactor (which a junior minister at the Energy Department, Dr John Cunningham, last week said was "imminent") and on the further development of the breeder, would not of themselves involve any commitment to a large additional nuclear programme. "Much more significant" decisions will be needed in a few years' time, it says. A Green Paper on energy policy later this year is expected to spell this out in greater detail.

An air of caution is further conveyed early on in the White Paper. International discussions on nuclear policy, it says, mean that the government is "not yet in a position to give a final response" to certain conclusions and recommendation from Flowers on the two "central issues" of waste and security. That more than anything else confirmed that in nuclear power politics, almost nothing is final. □

Lords judgment

EUROPEAN policies are never easily come by, least of all in energy. With the next chance for the Nine to take a step towards a common policy coming on 14 June, when the EEC Council of Energy ministers meets, more worried voices have added a note to the chorus of concern that normally prevails.

It comes from the House of Lords, whose Select Committee on the European Communities is one body in Britain that seeks actually to dissect the stream of proposals and consultative documents emanating from Brussels. Last week the committee released the latest example of its valuable work—its 28th report—to coincide with a debate in the House.

Coming from sub-committee F, which deals with energy, transport and research, the report examines three energy documents prepared by the Commission for the Council of Ministers. One compared EEC objectives set in 1974 with member states' forecasts of their own production and demands; the second warned of the lack of progress towards the aim of greater self-sufficiency; the third focused on the coal industry.

The committee's criticism is almost unsparing. No reliable conclusion can be drawn from the Commission's statistics, it says. There are unexplained inconsistencies and omissions in the figures. Important assumptions are insufficiently discussed, let alone justified. And the figures are not clear and persuasive enough to encourage the Council to take any important decisions, and could even provide an excuse for inaction.

The committee considers that the Community's attention should be concentrated "less on objectives and forecasts and more on the successes and failures of national policies, on the reasons for them, and on research into means of improvement, both technological and social".

One problem may be a surfeit of analysis and of discussion. As Lord Sheffield, a former head of the UK Atomic Energy Authority, commented in debate last week: "While the clock ticks it is difficult to find much that is new to say about the problem until something is actually done about it".

Chris Sherwell

IN BRIEF

OTS delay

Last week it was announced that the launch of the European Space Agency's (ESA) orbital test satellite (OTS), designed for experiments in communications, would not go ahead as scheduled next month because of damage to the American Delta 3914 launch vehicle. In April, ESA's geostationary scientific satellite, Geos, was thrown into a wrong orbit because the Delta 2914 rocket which launched it failed to separate according to plan.

The launch of OTS had already been delayed by the enquiry into the mishap over Geos. The damage to the Delta 3914 occurred when one of the nine solid fuel booster rockets attached to the launcher base fell off, damaging the rocket's first stage. The rescheduled launch date is 8 September; the Meteosat satellite, which was to have been launched on 15 September, has also been rescheduled, for 3 November. ESA decided that it was prefer-

able to delay both satellites by a few months.

OTA director resigns

Emilio Daddario, the first and only director of the Congressional Office of Technology Assessment (OTA), submitted his resignation last week. In a letter to Senator Edward Kennedy, chairman of the joint committee that controls OTA, Daddario noted that the fledgling office has now completed several major studies and he pointed out that he had long expressed a desire to leave as soon as OTA was firmly established. Established four years ago to provide advice to Congress on technical issues, OTA has encountered a difficult problem in giving long term advice to a body whose attention span is largely governed by the two-year election cycle. It has had a mixed reception and last month Kennedy asked the OTA's Independent Advisory Committee to con-

duct a thorough analysis of the office's role and operations. There have been rumours that Kennedy initiated the study and engineered Daddario's resignation as a prelude to taking firm control of OTA. Such rumours were rife when OTA was first established and Kennedy was made chairman of its Congressional board, but they have since proved groundless.

SRC support for AGT

The UK Science Research Council (SRC) is to increase its support for basic research relating to Advanced Ground Transport (AGT). A UK government White Paper released last week says the SRC hopes to spend up to £200,000 a year, concentrated mainly in the universities. This is more than the sum that the SRC has spent altogether over the past three years, but will be less than that spent from all sources over the period 1973-76; this totalled over £1 million.

An elderly visitor carrying baggage to his bedroom in the usual porterless hotel in modern Britain is likely to suffer at least minor abrasions as he wrestles with the heavily sprung fire doors which obstruct the corridors every few yards. The cost of installing these precautions has meant that hotel charges have risen much more rapidly than inflation alone would have dictated, and many cheaper hotels have closed.

It is right that we should try to make all our activities safe, and new laws may sometimes be required. There have been tragic hotel fires, some buildings are unnecessarily dangerous, but many modifications serve little purpose except to make work for inspectors and builders. The British Health and Safety at Work Act of 1974 contains useful provisions, but its application to laboratories is often governed by dogma rather than commonsense. Precautions, costly in time and money, are taken against the most unlikely catastrophes. Little research has been done to identify the probable dangers in different types of laboratory, where quite different work is done. Our Research Councils must be spending more than £2 million a year, out of their meagre funds, on 'safety'.

This is all part of the 'nannying' to which we are increasingly subjected. We see those who spend their holidays rockclimbing or even in that most dangerous of sports—motorcycling—insisting on following quite different rules when at work. The various admirable and tough outdoor

activities for young people described in his broadcast by the Prince of Wales, and to be supported by the Queen's Jubilee Appeal, will surely

Dangers of safety



KENNETH MELLANBY

be regulated out of existence.

I find most ludicrous the proposals to set up working parties to draft codes of practice for field work. Field work covers many different activities, some of which may be dangerous, and where obvious and well established precautions should be observed. But for the ecologist it is seldom more hazardous than a gentle country walk to observe birds or to pick wild flowers. Those of us who have done this sort of work should be grateful that someone actually

paid us, for many of our less fortunate friends do these things, possibly less systematically, when on holiday. I expect the code will tell us to change our socks if they get damp, and to wrap up well in cold weather. It is unlikely that any joker will be able to produce 'spoof' regulations which will be more absurd than those to be promulgated by the authorities.

We avoided this nonsense in the past because most field work was done almost entirely by responsible scientists themselves, walking on their own feet and observing with their own eyes. Today there is a growing tendency for scientists to remain indoors in their 'offices' and send assistants, admirable workers no doubt, but not personally committed to the investigation, to do the field work. The results of this practice are often, scientifically, disappointing, and it is understandable that the assistants' trade unions should be obsessional about their safety particularly when this increases opportunities for employment.

However, even the best schemes may have unfortunate results. On what was, by coincidence, the first day of European Farm Safety Week an estate worker drove his tractor a little too quickly over a 'sleeping policeman' (a ramp to slow traffic and prevent accidents). He bounced into the air, and hit his head on the roof of his newly-fixed safety cab, so that he had to be taken to hospital to have his scalp stitched. Too many safety precautions can be jolly dangerous.

news and views

An induced birth for the Solar System?

from M. G. Edmunds

DID the Solar System, like the Universe, begin with a bang? The idea that the supernova explosion of a star initiated the formation of the Solar System has recently been re-explored by A. G. W. Cameron and J. W. Truran (*Icarus* **30**, 447; 1977). The model they discuss may also have interesting consequences in deciding what astrophysical objects liberate significant amounts of elements formed during intense neutron irradiation.

The motivation for their renewed interest is an attempt to account for certain radioactive isotope abundance ratios in the early Solar System, as determined from chemical analyses of meteorites. Particularly important is the unusually high abundance of ^{26}Mg found in aluminium rich minerals in the Allende meteorite by T. Lee, D. A. Papanastassiou and G. J. Wasserburg (*Astrophys. J. Lett.* **211**, L107; 1977). The high magnesium isotope abundance implies that its rapidly-decaying progenitor ^{26}Al had been produced not more than a few million years before the condensation of solid bodies in the Solar System. The extreme temperature and pressure conditions of the supernova explosion of a massive star are the site of synthesis of heavy elements. Cameron and Truran therefore argue, since the unrelated chance occurrence of a supernova just before the Solar System formed is unlikely, that the aluminium synthesising supernova must have induced the formation. A shock wave from the supernova explosion passes through the interstellar medium, and this extra pressure on a cool region of somewhat higher density than average, might be sufficient to drive the region into a state of gravitational collapse. This collapsing cloud would then form the solar nebula out of which the Sun and planets formed. Nuclei in the expanding supernova remnant would mix with the cloud material, and by considering isotope ratios, Truran and Cameron estimate (with a large uncertainty) that perhaps 2% of the heavy elements—anything

from carbon onwards—in the Solar System came from the nucleosynthesis zones of the triggering supernova.

The supernova material could be delivered in either gaseous or solid form. Cameron now accepts that solid grains may well condense out of the synthesised heavy elements as the remnant expands and cools. Thus the radioactive nuclei could be incorporated into grains and directly transported, with their products as they decay, into the Solar System material. Subsequent heating of the grains during or after their incorporation into larger solid bodies may well have caused some chemical differentiation and loss of individuality, so that the supernova grains may not be identifiable with particular inclusions in the meteorite. Lee *et al.* give evidence that the inclusions containing high ^{26}Mg must have crystallised when both freshly synthesised ^{26}Al and older material were present, implying that the synthesis must have occurred only shortly before the solidification of the meteorite. Although the ^{26}Al could have become incorporated into grains, the grains must have been freshly made and then melted during or after meteorite formation. The mineral evidence seems to make it unlikely that the ^{26}Mg could have been brought in by old supernova grains long after its production by decay of ^{26}Al , and then directly incorporated as single grain inclusions. Production of ^{26}Al by processes other than supernova synthesis is unlikely, since those which have been proposed predict other isotopic anomalies which are not observed.

Apart from triggering the collapse of the protosolar cloud, the influence of the supernova on the formation of the planets would also be felt in the effect of decay of ^{26}Al as a heat source. Lee *et al.* suggest that the cores of any solid bodies greater than a few kilometres in radius would be melted while the aluminium was still radioactive.

Having a supernova just before the

Solar System formation is not without its embarrassments, since the model requires a revision of earlier ideas on the use of some other isotope ratios as indicators of the time elapsed between formation and the last nucleosynthetic event which contributed material. For example, it is assumed that ^{127}I and ^{129}I are produced in the r-process, in which nuclei capture neutrons in a flux strong enough to give insufficient time for β -decay of the intermediate product nuclei before the addition of the next neutron. In this process ^{129}I and ^{127}I should be produced in about equal amounts. Now ^{129}I is unstable, and the $^{129}\text{I}/^{127}\text{I}$ ratio at the formation of solid bodies in the Solar System used to be used as an indicator of how long the ^{129}I had been decaying since it was produced in an r-process event. The experimental abundance ratio of 10^{-4} implied that the last ^{129}I was synthesised as long as 10^8 years before solidification, obviously long before the ^{26}Al synthesis. This implies that the event which made the ^{26}Al could have released very little r-process material to the interstellar medium. Truran and Cameron propose an alternative in suggesting that all the ^{129}I observed in the Solar System was synthesised in the ^{26}Al -producing event, but by a process involving slow neutron capture to reduce the production ratio by two orders of magnitude. If 2% of the supernova material were incorporated in the Solar System, this would then give the experimental abundance ratio.

This removes the constraint that the last event which contributed r-process nuclei to the Solar System occurred about 10^8 years before formation. It could then have occurred much longer before, since it is no longer required to synthesise the ^{129}I . A similar argument applies for ^{244}Pu . The attraction claimed by the authors is that the ejection of r-process material from

M. R. Edmunds is in the Department of Applied Mathematics and Astronomy at University College, Cardiff.

supernovae can now be regarded as a very rare event. This is important in considerations of the removal of r-process elements from where they are formed near the core of a supernova, before they get trapped in a condensed neutron star or black hole remnant. The most plausible mechanism invokes perturbation of the supernova structure by strong magnetic fields during the collapse of the core. The difficulty is that magnetohydrodynamic calculations indicate that a very large amount of r-process material must inevitably be liberated, and if this occurred in every supernova then the Galaxy would be inundated with far too many r-process nuclei. The previous argument against this mechanism was that, since by a 'Cosmological Principle' the Earth is supposed to occupy no special

position in the Galaxy, it was unlikely that a rare r-process event occurred not very long before formation. This can be relaxed, since there is now no time constraint on when the last r-process contributing event occurred.

The idea that the Solar System origin was induced by a supernova shock wave is not likely to prove unpalatable, since a similar mechanism of cloud collapse induction is believed to cause the formation of bright massive stars in the spiral arms of the Galaxy as the result of the passage of a propagating density wave in the interstellar medium. Nucleosynthesis and Solar System-formation theorists may be rather more reluctant to throw away their useful, but perhaps misleading, timescales previously derived from some of the isotopic ratios. □

Optimal life-history strategies

from Robert M. May

THE idea that an organism's life-history strategy—its patterns of mortality, fecundity and parental care—depends on its physical environment and on its interactions with other organisms dates

back at least to Darwin and Wallace.

Stimulus for a more quantitative approach to the subject came from the work of people such as Deevey (*Q. Rev. Biol.* 22, 283; 1947) and Cole

(*Q. Rev. Biol.* 29, 103; 1954). On the empirical front, Deevey showed how data from natural populations of animals could be used to construct actuarial tables of age-specific survivorship and fecundity, similar to those constructed by insurance companies for human populations. On the theoretical front, Cole made the remark, which at first glance may appear paradoxical, that a population of immortal organisms, each of which produces N offspring each year, has the same growth rate as a population of organisms of which each lives for one year and then dies after producing $N+1$ offspring. The proof of this result, and its generalisation to somewhat more realistic situations, is trivial (although the simplicity is often concealed in thickets of algebra). So long as the survival probability is independent of age, and all organisms reproduce each year, then adults and juveniles have the same potential for future reproduction and 1 adult plus N offspring is reproductively equivalent to $N+1$ offspring. By extension of this argument, if the probability of a juvenile surviving the first year of life is smaller than the adult survival probability by a factor p , then $1/p$ additional offspring are required if the 'annual' life-history strategy is to have the same population growth rate as the 'perennial' one.

More recent studies have emphasised that the number of offspring, and their chances of surviving to maturity, depend on the amount of parental investment (for example, on the number and size of eggs, and on the amount of care, if any, given to offspring), and that this in turn influences the parent's survival probability. To quote one of a multitude of examples, Loschiavo (*Canad. Ent.* 100, 86; 1968) has demonstrated an almost inversely linear relation between the number of eggs laid and the longevity of the beetle *Trogoderma parabole*. Stearns (*Q. Rev. Biol.* 51, 3; 1976) catalogues other examples. Thus in any attempt to calculate optimum life-history strategies for particular environmental circumstances, it must be acknowledged that age-specific mortality and fecundity schedules are not independent, but are intertwined in a complex way; the trade-off between reproduction and 'personal growth' made by an adult in any one year will affect its probability of surviving that year, and thereby influence the possible trade-offs in all future years.

An excellent review of most work in this area up to 1975 has been given by Stearns. Schaffer (*Ecology* 55, 291;

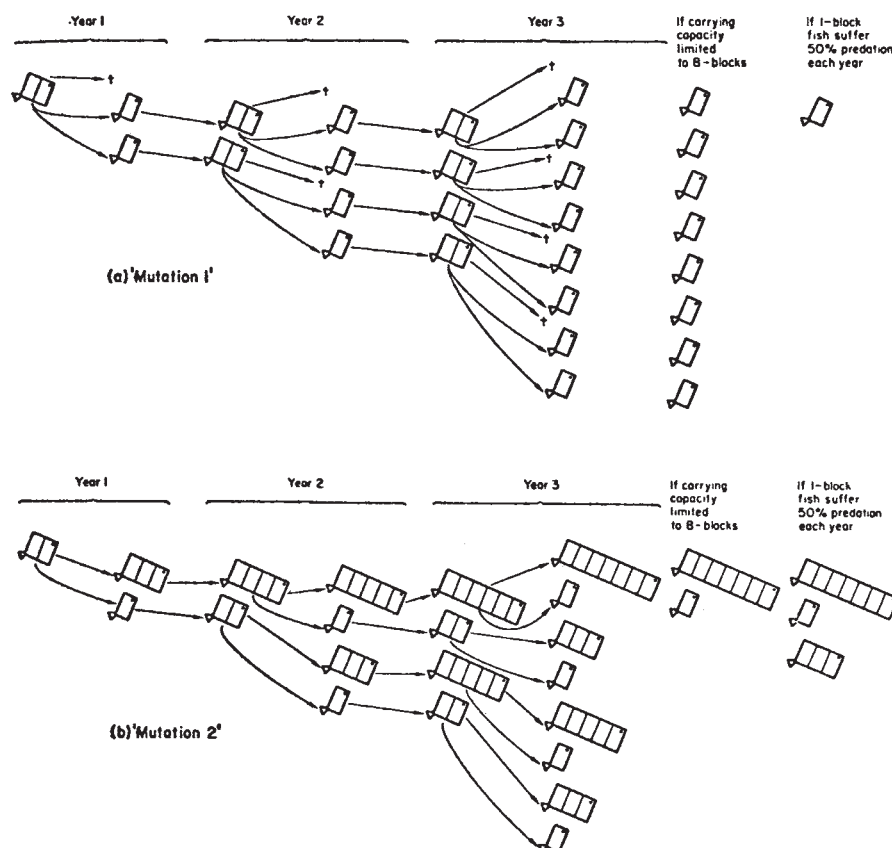


Fig. 1 The outcome of two different strategies for allocating resources in a hypothetical animal, the block-fish. *a*, Mutation 1 in which summer productivity (two blocks) is entirely allocated to reproduction; *b*, Mutation 2 in which one block of summer productivity is allocated to reproduction and one block to growth and, hence, to adult survival. (After Southwood, *op. cit.*)

Robert M. May is the Class of 1877 Professor of Zoology in Princeton University.

1974; *Am. Nat.* **108**, 783; 1974), Goodman (*Am. Nat.* **108**, 247; 1974) and others have given mathematical discussions, and Pianka and Parker (*Am. Nat.* **109**, 453; 1975) have subsequently given a graphical analysis of the way optimal strategies are moulded by trade-offs between present and discounted future reproduction. A discussion which is particularly simple and clear, yet which retains all the biological essentials, is due to Southwood (Ch. 3 in *Theoretical Ecology*, Blackwells (UK) and Saunders (USA), 1976). He invokes an imaginary animal, the parthenogenetic block-fish, which has a productivity of two blocks each summer and one block each winter. The two summer blocks can be added to the fish itself, or used in reproduction, in any proportion (any fish that does not add to itself dies); the one winter block is necessarily added to the fish itself. What is the optimal life-history strategy for the block-fish? Obviously there is no unique answer, but rather it depends on the environmental setting. Figure 1 illustrates the genealogy of two mutant strains of the block-fish: mutation 1 puts all its summer productivity into reproduction; mutation 2 puts only half its summer productivity into reproduction. If half the juvenile one-block fish are killed by predators each year, mutation 2 does better. Conversely, if the carrying capacity of the fish's environment is limited to a total of eight blocks, mutation 1 is represented at the end of three years by many more individuals, and is better placed to "bounce back" from adverse environmental fluctuations. Other assumptions could clearly be explored; for example, block-fish over a certain size could exploit additional resources, or mortality could be higher or lower for larger block-fish, or the environmental carrying capacity could be subject to random variations.

The insights provided by his imaginary block-fish, and by earlier work, enable Southwood to draw together a great deal of information about the trend in life-history strategies manifested by animals and plants, and particularly by birds and by insects. It would be nice to have more experiments aimed at explicit determination of the trade-off between present reproduction *versus* survival and future reproduction. Some such work exists, mainly directed at the relative investment between reproductive and vegetative parts in plants (see for example, Harper & Ogden *J. Ecol.* **58**, 681; 1970; Gadgil & Solbrig *Am. Nat.* **106**, 14; 1972), but by and large it is easier to issue hortatory calls for such experiments than to do them.

Of particular interest is the study of

the optimal reproductive strategy for eusocial insects given by Macevicz and Oster in the new journal *Behavioural Ecology and Sociobiology* (**1**, 265; 1976). They begin by observing that, for colonies of eusocial insects with an annual life cycle, the optimisation problem is to find the allocation of colony resources that maximises the total number of reproductives produced by the end of the colony cycle. For simplicity, Macevicz and Oster assume only two castes, workers *W* and reproductives *Q*. Differential equations are constructed to describe how the growth in the number of workers and of reproductives at any given time depends on *W* and *Q* themselves, on the resources *R* gathered by the workers, and on the 'control variable' that measures the fraction of resources devoted to producing workers, rather than reproductives. A third equation relates *R* to the ergonomics of the colony (the time and energy budgets of foragers); such a relation has been developed and compared with data by Oster (*Am. Nat.* **110**, 215; 1976), but Macevicz and Oster are content to use a simple phenomenological equation. Standard techniques from optimal control theory are then used to find the strategy that maximises *Q* at the end of the cycle. Although presented lucidly and unpretentiously, all this is an order of magnitude more sophisti-

cated than previous work on optimal life-history strategies.

The solution, which emerges independent of many of the details, is a so-called 'bang-bang' control policy. In the first phase, the total reproductive effort is directed towards producing workers. Then, at a critical time t_s , approximately one generation time before the season ends, the entire effort is thrown into producing reproductives (queens and males). The theoretical optimal control solutions are confronted with data on the wasp *Vespa orientalis* and on the hornet *Polistes fuscatus*. In both cases, the discontinuous 'bang-bang' policy is evident, and theoretical and actual values of t_s agree to within about 1 day. For *V. orientalis*, the observed and predicted number of workers and reproductives are in remarkable agreement over the entire colony cycle; for *P. fuscatus* the agreement is only fair.

Macevicz and Oster point out that discontinuous 'bang-bang' control policies will characterise a wide class of related optimisation problems, and, in particular, that such policies necessarily apply to any colony or population that undergoes exponential growth throughout its cycle. Realistic complications, such as non-linear density-dependent effects or time delays, may or may not lead to smoothly varying control. □

Superfluid helium: untying the tangle

from P. V. E. McClintock

THE celebrated superfluid properties of helium-4 only exhibit themselves while the liquid is being treated fairly gently. Up to a certain critical velocity it can flow through a tube in a completely frictionless manner, without needing to be pushed to keep it moving. At higher speeds, however, this simple superfluid behaviour disappears abruptly as turbulence is generated and a corresponding flow resistance appears. The resultant turbulent state of the superfluid is of considerable intrinsic interest, particularly in view of the quantum properties of the liquid. A recent paper in *Physical Review Letters* (**38**, 551; 1977) by K. W. Schwarz of the IBM Research Centre at Yorktown Heights represents the first serious attempt to tackle the dynamics of the turbulence and, judging by comparisons with experimental data, his theory seems to be remarkably successful.

Surprisingly, turbulence in superfluid helium is very much simpler than in other liquids, mainly because the so-called quantisation of circulation means that the turbulence takes the

form of a mass of vortex lines such that any element of vortex line is identical to any other. Each vortex line consists of a narrow core region, which may be imagined as being hollow, surrounded by superfluid which circulates rapidly close to the core and more slowly at large distances from it. Turbulence consists, then, of a seemingly random tangle of these vortex lines in constant relative motion because each element of line exists in the flow fields around all the other lines. The situation has been likened to a mass of moving spaghetti, or a basket of writhing snakes (although free ends would be unstable, so one is really obliged to imagine spaghetti in tangled rings, or snakes which have swallowed their tails). These analogies must be treated with caution, however, because in reality the spacing between neighbouring segments of line is very much greater than would seem to be implied.

Most of our present understanding

P. V. E. McClintock is a lecturer in the Department of Physics at the University of Lancaster.

of the turbulent state is due to the work of W. F. Vinen (*Proc. R. Soc. A* **240**, 114, 128, 1957; *A* **242**, 493, 1957; *A* **243**, 400, 1958). He envisaged that, in the equilibrium situation, the rate at which new vortex line was being created by 'frictional' effects would be exactly balanced by an annihilation mechanism whereby vortex line would be being continually destroyed. His theory, which was based largely on dimensional considerations, had nothing to say about the details of either the creation or the annihilation mechanisms, so that there has been a continuing debate about what might actually be going on in the liquid. One school of thought held that the lines were annihilated when, in the course of their random movement, they crossed each other, while others believed that such a crossing would be able to occur without the lines even 'noticing' each other, let alone being an event which would lead to their mutual destruction. Sitton and Moss (*Phys. Rev. Lett.* **29**, 542; 1972) suggested that the annihilation mechanism arose from the Magnus force which tends to drive together parallel sections of line with opposite circulation; but this idea was not supported by subsequent experimental work by Ashton and Northby (*Phys. Rev. Lett.* **30**, 1119; 1973). The situation has remained obscure.

There have, in fact, been growing indications of the inadequacy of the Vinen model, arising mainly through experiments in which charged ions are used to probe the movements of the tangle of vortex lines. In one such experiment, Ashton and Northby (*Phys. Rev. Lett.* **35**, 1714; 1975) were able to demonstrate that the tangle was not moving at the same speed as the superfluid (in fact, it was travelling in the opposite direction), thus contradicting one of the central assumptions of Vinen's theory, that the tangle of lines must be isotropic. It was clear, therefore, that a more sophisticated approach was needed.

What Schwarz has now attempted is to take some explicit account of the actual way in which the lines move relative to each other and to distant parts of themselves: a daunting task which has, of course, required a statistical approach. His central equation, describing the rate of change in the length of line per unit volume of liquid, is extremely complicated, but contains no adjustable parameters. An interesting feature is that it seems to imply, quite contrary to the premises of earlier theories, that the most important velocity is not that of the random inter-line movements, but is that of a segment of a line relative to other segments of the same line. The solution

of this equation has necessitated the use of numerical methods. It turns out that, for any given temperature and flow velocity of the liquid, a forward integration in time leads to the same steady-state distribution of vortex line, regardless of the assumed initial distribution of lines: apparent confirmation that the theory is at least self-consistent. More importantly, it seems to be in remarkably good agreement with experiments. Contrary to the Vinen theory, it predicts that there will indeed be a relative velocity between the vortex tangle and the superfluid, with the tangle tending to move in the same direction as the thermal excitations in the liquid. Furthermore, both the magnitude of this velocity and its temperature dependence are in good agreement with the measurements of Ashton and Northby. The predicted line density turns out to be in satisfactory agreement with Vinen's original measurements; and, again, the calculated temperature dependence is as it should be.

These results are extremely encouraging. It will be interesting to see whether the model will be able to deal equally successfully with critical velocities for the onset of turbulence, and with the transient situations which occur when turbulence is building up or dying away in the superfluid. No doubt there will be a brisk renewal of interest by experimenters, now that there is a seemingly satisfactory theory waiting to be verified or contradicted.

What can artificial intelligence offer psychology?

from Tim O'Shea

A meeting entitled 'What can artificial intelligence offer to psychology' was held at the University of Bristol on 14-15 April, 1977. It was organised by Richard Young (University of Edinburgh) and Tony Conway (University of Bristol) for the Society for the Study of Artificial Intelligence and Simulation of Behaviour (AISB).

ARTIFICIAL Intelligence is an investigation into the principles underlying actual and possible forms of intelligent behaviour. In part this objective is shared with psychology which is, however, concerned with human and animal behaviour. The methodology of artificial intelligence, unlike that of psychology, is based not on experimentation with people and animals, but

on the design, implementation and analysis of computer programs which exhibit intelligent behaviour. The aim of the meeting was to examine the relevance and applicability of this methodology to psychological research. Most of the speakers were active research workers in artificial intelligence but many of the other participants were cognitive psychologists. The work presented ranged in application from H. C. Longuet-Higgins' (University of Sussex) program for the perception of melodies to S. Weir's (University of Edinburgh) account of therapeutic work carried out with an autistic child.

Opening the meeting R. L. Gregory (University of Bristol) used the variety of types of intelligent behaviour as the basis for criticising attempts to measure intelligence by a single numerical IQ score. He proposed a distinction between 'design intelligence' (a pre-existing or 'wired-in' intelligent solution to a problem) and 'processing intelligence' (the generation *de novo* of intelligent solutions) and argued that artificial intelligence has made explicit the large amount of 'processing intelligence' necessary for perception and simple skills. He also suggested that the construction of robots with restricted 'design intelligence' provides a way of examining the effective use of limited data.

A large proportion of the talks described fully or partially implemented computer programs. These papers were probably the most useful, as the research methods of artificial intelligence and the current state of the art were made explicit through the discussion of the actual programs. The thesis that artificial intelligence should be regarded as theoretical psychology was cogently put by Longuet-Higgins. He argued that artificial intelligence provides psychologists with an appropriate descriptive medium for the expression of theories. These take the form of computational mechanisms which can be used to specify the complex interacting processes necessary for intelligence. This view of artificial intelligence as theoretical psychology was strongly supported by R. Schank (Yale University) in his discussion of language comprehension. Schank argued that process models are essential in any account of linguistic phenomena and that such models could best be realised as computer programs. He illustrated his argument with an account of a computer program which takes as input stories set in restaurants. It uses 'scripts' of the expected sequences of

Tim O'Shea is a Research Associate in the Department of Artificial Intelligence at the University of Edinburgh and Editor of the AISB Newsletter.

actions in restaurants to 'understand' such a story. The extent of its understanding is demonstrated by its ability to answer English questions about the story, including making inferences about events that must have occurred between the incidents specifically mentioned.

Many speakers argued that the construction of computer models which exhibit intelligent behaviour requires the use of knowledge from a variety of different domains. For example, phonetics, syntax, semantics and pragmatics are all necessary for the synthesis of intelligible speech. This point was nicely illustrated by S. Isard (University of Sussex). He is working on the problem of determining the 'right' intonation contour for a given English sentence taking into account the intended meaning and the context in which it is spoken. Consider the sentence 'I bought a boat and *that* sank'. A different intonation pattern is applied in the case where the speaker assumes it is known that his first boat blew up, from the pattern applied where the speaker is trying to correct the mistaken impression that it was his first boat that sank. Isard described a program which he is developing to explain how the differing contexts in which a sentence is uttered give rise to different intonation patterns. The program produces spoken English generated by a speech synthesiser. (The tape-recordings of the generated speech he played had an oddly Scandinavian lilt.) One argument for the development and specification of psychological theories as computer programs was that they can then be run on a computer and their consequences seen in a clear form. Isard illustrated this by describing various experimental changes he had made to the components of his program and discussing the effects these changes had had on the intonation of the generated speech. He concluded his account by observing with disarming equanimity that 'parts of my program are starting to interact in ways that are making me uncomfortable'.

How best to organise such complex programs remains an open question. A. Sloman (University of Sussex) argued that there was a need to deploy the different components of an intelligent program concurrently rather than sequentially. Sloman illustrated his argument by a description of the interactions between the different levels of a program which interprets visual scenes composed of overlapping letters. Such arguments for the need for parallel processing in computer models of intelligent behaviour were responded to with enthusiasm by some of the psychologists present. But on the whole the psychologists were sceptical of compu-

tational analyses of cognitive functions which were not based on any concrete computer program. The main criticism ironically enough was one often levelled at psychological theories, namely a lack of rigour together with a resulting absence of predictive power. P. J. Hayes (University of Essex) argued that the difference between theories in artificial intelligence and those in psychology is that the former must be implemented as computer programs to be viable, while the latter must make experimentally testable predictions. J. M. Brady and R. Bornat (University of Essex) asserted that the main strength of computer programs as models of cognitive processes was that they were precise enough to be refuted. They contrasted such programs with contemporary models in cognitive psychology which they suggested are couched in computational terms but are not, in practice, implementable as computer programs. D. A. Allport (University of Reading) gave examples of general organisational principles such as heterarchy and debugging which might be applicable in psychological explanations. However he argued that the main benefit of artificial intelligence to psychology so far lies in the carefully detailed analysis motivated by the demands of developing computational descriptions rather than in the application of general principles. He criticised workers in artificial intelligence for paying insufficient attention to known psychological phenomena. While this criticism certainly applies to some of the work presented it could not, for example, be applied to G. Kiss's (University of Warwick) analysis of the computational complexity of some simple cognitive processing tasks. This analysis was directly applied to experimental findings on memory span and other results. Nor could this criticism be applied to the style of development espoused by G. Luger (University of Edinburgh) for a computer program which solves 'A' level mechanics problems. Luger repeatedly emphasised the necessity for analysing protocols of human subjects as a source of design ideas for programs intended to have natural language understanding and problem solving capabilities. Allport also pointed out that much artificial intelligence work is judged against the intuitive sufficiency of the mechanisms used. The validity of this objection was made clear by the ambiguous and easy response of many of the speakers when questioned on their views of computer programs which performed some task requiring intelligence but did not make the same errors as humans.

Despite the gladiatorial format of the meeting there was a surprising degree of consensus between the psy-

chologists and the workers in artificial intelligence. This was particularly apparent during M. Eisenstadt's (Open University) account of a new course in cognitive psychology. This course is based on the artificial intelligence view that representation and symbolic processing are central to the specification of cognitive functions. No one seriously questioned that artificial intelligence is in principle useful to psychology and there was general agreement that there is a large gap between current experimental work in psychology and the construction of computer programs. The difficulty of making empirically testable predictions of human performance (from the execution of computer programs) was repeatedly stressed. In the long term the most valuable contribution of artificial intelligence to psychology may be to liberate it from a methodology based on a quest for simple explanations relating quantitative variables. This search for simple mechanisms underlying human behaviour is characterised by the collection of a mass of experimental data in the absence of precise theories about the underlying cognitive functions. Artificial Intelligence offers a methodology for the development and elaboration of symbolic representations for the processes underlying intelligent behaviour. □

Probing satellite DNA

from Gabriel Dover

THE point has been reached in the study of highly repetitious (satellite) DNA of higher organisms where earlier plausible generalities can no longer significantly advance our understanding of the origin and function of this species of DNA. These generalities were usually based on correlations between aspects of chromosome behaviour (often during meiosis) and the composition, amount and chromosomal location of heterochromatin (or satellite DNA).

Early studies showed that the majority of satellite DNAs are highly homogeneous in sequence and some can differ from each other (whether in the same or different species) by relatively few base changes despite the lapse of long periods of evolutionary time. They are concentrated in sections of the chromosome adjacent to the centromeres and telomeres which are involved in chromosome positioning

Gabriel Dover is a lecturer in the Department of Genetics in the University of Cambridge.

during the cell division cycle. Furthermore there is no concomitant replication of satellite DNA in specialised cells having polytene chromosomes.

These almost diagnostic features of satellite DNA have led to much current interest in the molecular mechanisms responsible for the generation and maintenance of homogeneity of sequence and in its biological role: a situation analogous to the conserved and non-conserved sequences, within and between species, of multigene families and their spacers.

Some answers to these questions can be gained from studies that probe more extensively the sequence organisation of satellites, the finer patterns of satellite distribution on the chromosomes, and the extent of satellite sequence homologies in species of known phylogenetic relatedness.

Important new data obtained by a combination of these approaches is reported on the satellite DNAs of *Drosophila melanogaster* (Brutlag *et al. J. molec. Biol.* **112**, 31; 1977; Shen & Hearst *J. molec. Biol.* **112**, 495; 1977). There are four major satellites in this species (Peacock *et al. Cold Spring Harbor Symp. quant. Biol.* **38**, 405; 1973; Endow *et al. J. molec. Biol.* **96**, 665; 1975) ranging from 2.6–5.3% of total DNA (equivalent to 4–8 million bases each) that are located at the chromocentres of salivary gland cells. If satellites are the basis for the differences in behaviour of pairs of homologous chromosomes it might not be unexpected to find a pattern of distribution of these four satellites that is chromosome specific, and in addition to locate sequence variants of individual satellites on individual chromosomes. Brutlag *et al.* after determination of the composition and properties of major and minor satellite peaks (resolved in antibiotic-CsCl gradients), have demonstrated that there is an upper limit of between 8–10 chromosomal sites for each block of satellite (of average size 750,000 bases) and that these blocks are predominantly interspersed with each other. Their argument is based on the interpretation that the minor peaks represent linkage molecules at the boundaries of contiguously covalently linked blocks and have buoyant densities commensurate with a dual satellite composition. Hybridisation of homogeneous pure satellites to a minor peak associated with satellite IV ($\rho = 1.705 \text{ g cm}^{-3}$) shows that it contains sequences of three flanking satellites (1.705, 1.686 and 1.672), in conjunction with some main-band DNA.

Although there remain several, as yet, untested minor satellites, this interpretation is secure when considered in conjunction with *in situ* hybridisation experiments at CSIRO (Peacock *et al.*,

in preparation), that have revealed chromosome-specific patterns of interspersed blocks of satellite making up the heterochromatin around mitotic centromeres.

The existence of sequence variants within a satellite can be demonstrated with the use of restriction endonucleases as probes to examine the range and types of long periodicities that may overlay the basic short repeating unit. If sites, susceptible to attack by a particular endonuclease, are regularly spaced throughout a block of satellite DNA then the DNA fragments produced after enzyme digestion will be of a uniform size indicative of this high-order spacing. Satellite IV has hitherto proved particularly resistant to a number of restriction enzymes and it has not been possible to detect higher-order periodicities of the sort reported in satellites of other organisms (Southern *J. molec. Biol.* **94**, 51, 1975; Botchan *Nature* **251**, 288; 1974) and in satellite III of *D. melanogaster* (Manteuil *et al. Cell* **5**, 413; 1975). However Endow (in the press) has recently succeeded in cutting it with *Mbo*II, confirming its repeating pentamer purine sequence of (AGAAG)_n and its high (95%) conservation of sequence.

The novel use of 4,5',8- trimethylpsoralen (trioxsalen) on satellite IV by Shen and Hearst in conjunction with denaturation microscopy has revealed a regular distribution of cross-linkable pyrimidine sites at intervals of approximately 250 base pairs and brings this satellite firmly into line with those of mouse, guinea-pig, calf and green monkey in its organisation. This is not only an important step forward in the application of a new method for probing satellite sequence organisation, but also raises intriguing questions concerning the universality of higher-order repeats, the mechanism of their evolution and maintenance and, naturally, their biological role. Recent data by Zachau and his colleagues at Munich on the organisation of mouse, guinea-pig and African green monkey satellite DNAs (Horz & Zachau *Eur. J. Biochem.* **73**, 383; 1977; Altenburger *et al. Eur. J. Biochem.* **73**, 393; 1977; Fittler *Eur. J. Biochem.* **74**, 343; 1977) indicates that satellite evolution is even more complex than at first realised. The pattern of fragment sizes produced after successive- and co-digestions of satellite DNA with several restriction enzymes revealed the existence of satellite sequences (segments) within the block of satellite DNA that are variants of a common ancestral sequence. This is with respect to the organisation of higher periodicity, in that sites sensitive to different restriction enzymes are clustered in different parts of the satellite block.

The stage is now set to investigate the possibility of equivalent segments (sequence variants) in the satellites of *Drosophila* and to relate this to the new data of Brutlag *et al.* and Shen *et al.* Sequence variants of a satellite could reside either on different homologous chromosomes (thus reinforcing the chromosome differences in the patterns of satellite distribution) or in other species that are closely related to *melanogaster* and that have some satellites in common. Such comparisons, currently underway by Sederoff and his coworkers in Oregon; at Canberra (Australia) by Peacock's group; and at Cambridge, UK on the *melanogaster* species group, might reveal something of the order of events in the evolution of satellite DNAs that has led to their contemporary composition and distribution both within and between closely related genomes and resolve some of the intriguing problems associated with this enigmatic species of DNA. □



A hundred years ago

"SUMMER Schools" are becoming a regular institution in America, and no more pleasant way could be devised of combining the *dulce* and the *utile* than that of a proposed aquatic summer school of natural history, which, under the direction of Prof. Theodore B. Comstock, of Cornell University, expects to charter a large steamer, and spend the summer around the shores of Lake Superior in the study of the geology and natural history of that region. The steamer will accommodate from seventy-five to one hundred passengers, to be made up of students and professors. Regular instruction will be given in the form of lectures during the voyage, and every facility afforded by the examination of mining localities and the like. The vessel will probably start from Cleveland or Detroit on July 7, and proceed thence to Lake Superior, making its full circumnavigation. The coast of Georgian Bay, on Lake Huron, will be investigated on the return voyage. The expense will probably amount to about 125 dollars for each person. In addition to the aquatic summer school mentioned above, Harvard University announces two special summer courses of instruction, one in zoology and the other in geology.

From *Nature*, **16**, 31 May, 92; 1877.

articles

History of the Mediterranean salinity crisis

Kenneth J. Hsü

Geological Institute, ETH, Zurich, Switzerland

Lucien Montadert

Institut Français du Pétrole, Rueil-Malmaison, France

Daniel Bernoulli

Geologisches Institut, Universität Basel, Basel, Switzerland

Maria Bianca Cita

Istituto di Geologia, Università Milano, Italy

Albert Erickson

Department of Geology, University of Georgia, Athens, Georgia

Robert E. Garrison

Earth Sciences Board, University of California, Santa Cruz, California

Robert B. Kidd

Institute of Oceanographic Sciences, Wormley, UK

Frederic Mèlierès

Laboratoire de Géologie Dynamique, Université de Paris, Paris, France

Carla Müller

Geologisch-Paläontologisches Institut, Johann Wolfgang Goethe Universität, Frankfurt, FRG

Ramil Wright

Department of Geology, Florida State University, Tallahassee, Florida

A history of geodynamic evolution of the Mediterranean leading to the salinity crisis is outlined, based on the 'desiccated deep-basin model'. An accurate portrayal of the crisis is presented, based on data from new drilling and studies of on-land geology.

THE discovery of the Mediterranean Evaporite by the drilling from Glomar Challenger in 1970 proved that salt exists under deep-sea floor¹, and demonstrated that giant salt deposits could have been formed within a relatively short geological time². The almost synchronous onset and termination of the Mediterranean salinity crisis implies catastrophic changes of environments in a region over two and half million square kilometers in extent. This fact did much to throw doubts on Lyell's substantive uniformitarianism³.

The significance of the Deep Sea Drilling Project (DSDP) discovery was, however, shadowed by controversies. That the Mediterranean Evaporite is Messinian (Late Miocene) in age seemed to be just about the only consensus after the Leg 13 drilling. Even the shipboard scientific staff could not reach an agreement on the genesis of this unusual formation. Chapter 43 of the Leg 13 cruise report presenting the desiccated deep-basin model (ref. 4) was authored by K. J. Hsü, Maria Cita, and W. F. B. Ryan because they were the only members of the shipboard staff totally convinced of its plausibility. Alternative interpretations were given by other shipboard staff in the Initial Cruise Reports and later in other publications^{5,6}. The reactions of the scientific community to the desiccated deep-basin model were also divergent. Favourable commentaries were not rare. On the other hand, many critiques were sceptical, or downright hostile.

The importance of clarifying the question of the Messinian salinity crisis did not escape the attention of the JOIDES organisations. A second cruise to the Mediterranean took place in 1975. We are glad to report that we reached a near-consensus on the previously controversial question of the origin of the Mediterranean Evaporite. This article is not a perfunctory report requiring a co-authorship by the shipboard scientific staff. It is a

unanimous opinion written by the senior author for ten co-authors, who represent all but two of the shipboard scientific staff during the Leg 42A cruise.

The Miocene Mediterranean

Whereas there seemed to be general agreement that the Messinian (Late Miocene) evaporites were deposited in shallow water conditions, controversy revolved around the basin depths at times of evaporite deposition⁷. The crux of the matter is the genesis of the Mediterranean basins. When did the Mediterranean basins form? In the Early and Middle Miocene before the Messinian salinity crisis? In the Pliocene shortly after the salinity crisis? Or "did the main phase of subsidence occur rather abruptly during the Pleistocene and Holocene"¹⁰. Syntheses of regional tectonic data led to the conclusion that the deep basins of the Mediterranean have evolved to a stage quite similar to their present configuration before the Late Miocene when the salinity crisis began^{4,11-14}. Of the five major Mediterranean basins, the Levantine and Ionian basins may be relic Tethys of Mesozoic age; The Balearic and Tyrrhenian were formed during the Early and Middle Miocene, after the culmination of the Alpine orogeny. Perhaps only the Aegean has undergone significant Plio-Quaternary subsidence.

Deep-sea drilling during the Leg 42A has unearthed convincing evidence that deep Mediterranean basins were in existence before the Messinian. Drilling at Site 372 (Fig. 1) indicated that the Balearic Basin was formed by rifting during the latest Oligocene or earliest Miocene time. Palaeobathymetric analyses on the basis of benthonic foraminifera estimated that this part of the Balearic margin was at least 900 m deep during the Early Miocene (Burdigalian) time. The water depth reached in excess of 1,200 m during the late Burdigalian and subsided gradually to a depth more than 1,500 m before the Middle Miocene ended¹⁵.

The pre-Messinian sequence at Site 372 is mainly hemipelagic, similar to the sediments deposited there today. The planktic and benthic faunas and floras are fully open marine. Aside from benthic foraminifera, psychrospheric ostracods also occur in some Early and Middle

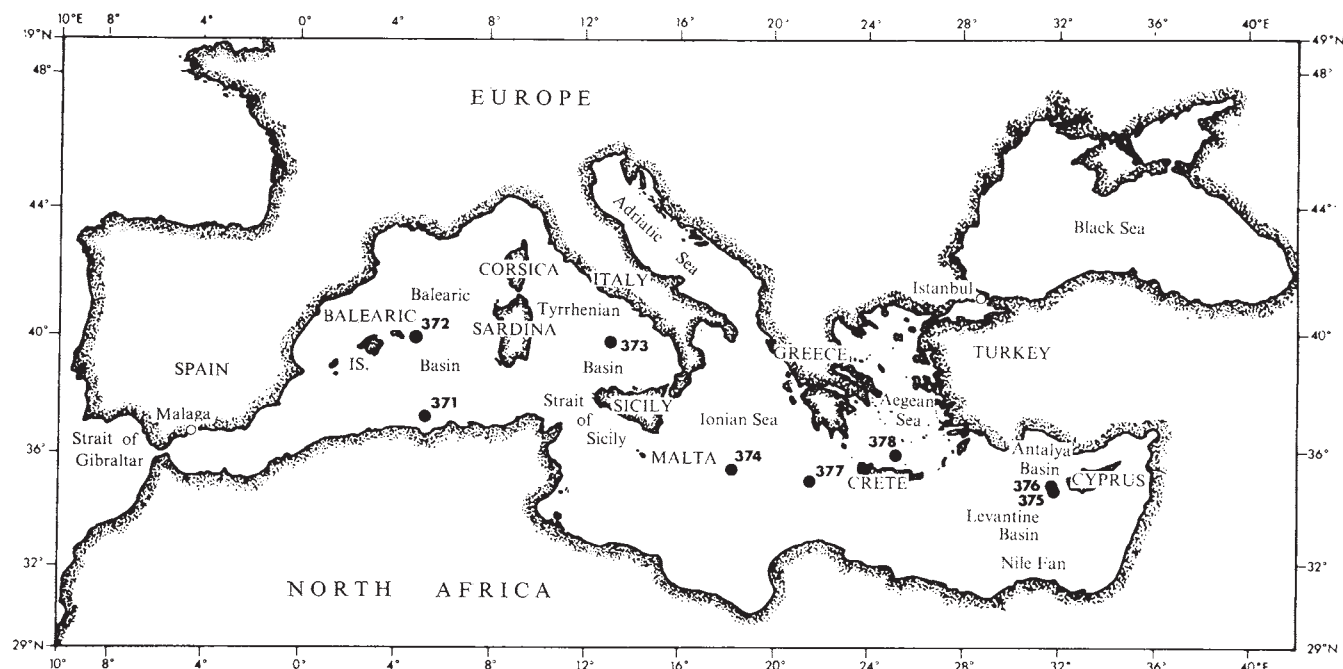


Fig. 1 Drill sites of DSDP Leg 42A.

Miocene cores, indicating water-depths in excess of 1000 m (ref. 16).

Drilling results at Site 373 seamount indicated that a deep Tyrrhenian Basin was also in existence during the Messinian. Palaeotemperature estimated for the submarine cementation (by calcite) of the early Pliocene pillow breccias at this site is estimated by isotopic evidence to be 6–8 °C (ref. 17). Such a low temperature implies that the volcanic seamount was deeply submerged then, only a short time after the salinity crisis.

We penetrated beyond the Messinian at Site 375 west of Cyprus in the Levantine Basin. As in Hole 372, the pre-Messinian sequence at this eastern Mediterranean site is also mainly hemipelagic. Palaeobathymetric analyses indicate that the Levantine Sea was a deep, open sea during the Miocene before the Messinian salinity crisis¹⁵.

Events leading to the salinity crisis

Geological reconstructions indicate the presence of an equatorial ocean, which separated Europe from Africa during the Jurassic and Cretaceous and the ocean was named Tethys^{14,18}. The connection between the Atlantic and the Indo-Pacific was maintained through a shallow seaway after the joining of the two continents in late Cretaceous¹⁹. This shelf sea started to disappear during the Burdigalian (late Early Miocene). This was one of the major palaeobiogeographical events of the Tertiary; it was during this period that "the present pattern of land and sea was established"²⁰. The joining of Europe and Asia permitted the exchange of land fauna between those continents, and started the differentiation of the Indo-Pacific and Atlantic-Mediterranean marine faunas. Whereas the evidence from vertebrate palaeontology indicates the establishment of first intercontinental connection between Eurasia and Africa during the Burdigalian²¹, the micro-palaeontological data suggest that there might have been repeated transgression into the Mediterranean from the Indo-Pacific till Middle Miocene time²² (Fig. 2).

The orogenic movements which fused Eurasia and Africa also raised new mountains in the Taurides, the Hellenides, the Dinarides, and eventually, somewhat later in the Middle or early Late Miocene, the Helvetic Alps. This series of

movements led to a separation of the Mediterranean and an eastern European inland sea, the Paratethys. Stratigraphical and palaeobiogeographical data indicate that the Burdigalian connection between the two seas went through the Peri-alpine Depression north of the Alps^{22,23}. This seaway was eliminated early in Middle Miocene, when the Alps rose and marine waters withdrew from the Molasse trough. A last thread of contact was probably maintained between the Mediterranean (Langhian) and the Paratethys (early Badenian) through an opening in northern Italy and north-western Yugoslavia. The complete separation of the two inland seas took place some 14 to 15 million years ago during the Serravallian (late Middle Miocene). The last Indo-Pacific flooding that invaded the Paratethys later in the Serravallian (14–13 Myr) did not reach the Mediterranean²².

The Middle Miocene separation had two consequences. The Paratethys could no longer receive marine water from the Mediterranean nor could the Mediterranean receive freshwaters from that part of Eurasian rivers which now emptied themselves into the Paratethys. When the connection was open, surplus ions resulted from evaporative excess in the Mediterranean may have found their way to the Paratethys, and were deposited as the lower Middle Miocene salts of the Pannonian and Transylvanian Basins²². After the loss of communication the Mediterranean was deprived not only of much freshwater influx but also of one of its means of the elimination of evaporative waste.

The Mediterranean's last links to the world ocean were the Betic and the Rif Straits. The deep Betic Strait shoaled after the end of the Middle Miocene; no Late Miocene psychrospheric ostracodes were found inside the Mediterranean¹⁶. Yet open marine conditions prevailed in the Mediterranean during the earlier parts of the Late Miocene (Tortonian and early Messinian)²⁴.

The Messinian change from normal marine to an evaporite-forming condition was a sudden event. The palaeobotanical record gives no evidence of an abrupt change in climate. There had been a general trend towards a drier and cooler climate since the Burdigalian, when the Mediterranean was first cut off from the Indian Ocean²⁵. Thus we cannot interpret the change from normal marine to evaporative conditions as a response to climatic factors; the

change had to be related to a final closure of the openings at the west during the latest Miocene. With the isolation and a serious hydrographic deficit, the salinity crisis of the Mediterranean finally became inescapable.

The Messinian salinity crisis

Seismic records suggested a twofold division of the Mediterranean Evaporite²⁸. (1) An Upper Evaporite sequence, with numerous reflectors, up to several hundred metres thick, and consisting of dolomite, gypsum, anhydrite, and some salts. (2) The Main Salt and Lower Evaporite sequence, seismically homogenous salt, up to a thousand metres thick or more, underlain by several evaporite reflectors. The distribution of the Main Salt unit is restricted to the more central part of each of the Mediterranean basins. The distribution of the Upper Evaporite unit is, however, more extensive.

Since drill cores only yielded information on the Upper Evaporite, we depend on land sections for an overview of the Messinian salinity crisis. The onset of the salinity crisis as in Sicily, for example, indicated by the contact of *Calcare di base* on 'Tripoli', signifies a very rapid change from a bathyal environment of hemipelagic sedimentation to a shallow, or subaerial environment of carbonate deposition and diagenesis²⁴.

The history of the early evaporite deposition can be deciphered from a reference to the section of the Cattolica basin, Sicily²⁷. The Lower Evaporite sequence there lies above the Tripoli Formation and includes (1) *Calcare di base*, (2) Cattolica Gypsum, (3) Clastic Gypsum beds, (4) halite and potash salts.

The salts are in general 300–500 m thick. Decima²⁸ divided a 330-m section of salts at Porto Empedocle into four units. The Unit A is a halite with laminae of anhydrite and is 108 m thick. The bromine content of the halite is about 400 p.p.m. The Unit B is well laminated halite, kainite, polyhalite and anhydrite, and is 115 m thick. The bromine content ranges from 200 to 250 p.p.m. except for the uppermost pure halite which contains some 100 p.p.m. Br. The Units C and D overlie the lower salts unconformably. They are 107 m thick and consist of halite, with anhydrite and anhydritic shales. The halite in these upper units has only traces of bromine (10 p.p.m. or less).

The lower salts with their high Br were precipitated from brines derived from evaporated seawater. In contrast the upper salts were recycled. Sedimentological studies²⁹ indicated that the upper salts were deposited in shallow saline environments. On the other hand, the well laminated Unit B, with sequences of cyclic lamination that can be correlated for long distances, were precipitated in a relatively deep subaqueous environment.

The Lower Evaporite is overlain unconformably by a Upper Evaporite of gypsum and marls. A comparison of the submarine Messinian with the Sicily section suggests a similar two-stage evolution of the Messinian salinity crisis in other Mediterranean basins. After an initial desiccation or partial desiccation, the Lower Evaporite was probably precipitated when the supply of brines was sufficiently continuous, to cause the accumulation of thick halite and potash salt deposits on the deepest floor of the Mediterranean basins. Toward the end of the Main Halite deposition, the supply of brine probably ceased because of a complete isolation from the Atlantic. The brine level was drastically drawn down because of evaporative loss. Large areas on the periphery of the salt basins were exposed. Waters from the continent or from local precipitation formed salt creeks, eroding primary salts (for example Units A and B, Porto Empedocle), and depositing secondary salts (for example Units C and D) in the centre of the salt basins. This desiccation period was represented by the widespread unconformity separating the Lower and

the Upper Evaporite units. Rivers cut canyons on the margins of the continents and some of these canyons were eventually flooded by the Upper Evaporite deposits²⁶.

The erosional event was ended by a new inundation of the Mediterranean, when the Mediterranean was probably again filled to the brim. The lower marine marls of Messinian age at DSDP Site 372 (Core 9/2) are the first sediments above the unconformity and they may have been deposited during this intra-Messinian flooding. The subsequent history of the Upper Evaporite sedimentation was probably characterised by periodic influx of marine waters into a largely desiccated deep basin³⁰. A repetition of marine inundation, followed by isolation, and evaporative draw-down led to the accumulation of marls, stromatolitic carbonates, gypsum, anhydrite and salts, in cyclic sequences.

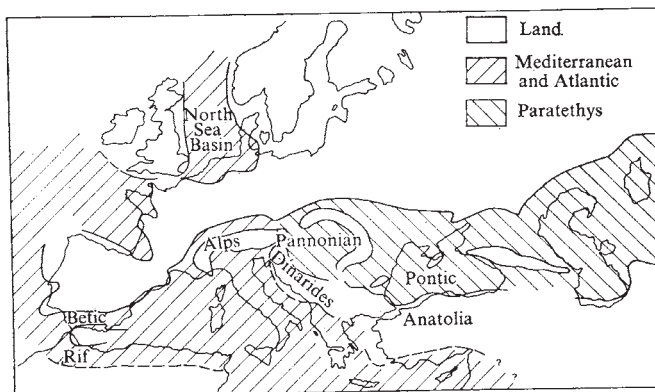
Lago Mare

The geological record of the eastern Mediterranean holes revealed a drastic change in depositional environment during the latest Messinian time before the final Pliocene marine inundation. After the last salt deposition of the Upper Evaporite, the Ionian and Antalya basins were almost totally dried up. Slight changes in the water-budget led to conditions of cyclic sedimentation. The very last Messinian sediments at Sites 374 and 376 are marls, however, deposited in a standing body of water. Isotopic evidence suggests a considerable influx of continental waters to those previously desiccated basins³¹.

Site 376 samples contain an *Ammonia-Cyprideis* fauna, indicative of the euryhaline environment. Similar *Cyprideis* and other euryhaline faunas are present in numerous uppermost Messinian sections on land in both the eastern and western Mediterranean countries. The Mediterranean hosted a series of fresh or brackish water lakes toward the end of the Messinian. We propose that the term *Lago Mare* be used to designate the latest Messinian lake (or oligohaline) environments in the Mediterranean, subsequent to the evaporite-deposition, but before the Pliocene marine sedimentation²⁴.

The change from salt-precipitation in shallow salinas to deposition of marls in *Lago Mare* occurred within a very short time interval probably in less than 100,000 yr. The change implies a radical alteration of the hydrographic budget. There was, however, no drastic climatic change during the Messinian. The environmental change must have been related to a reorganisation of the drainage system in Europe. The sudden appearance of so much fresh or brackish water, together with a typical Paratethyan fauna, probably indicates a sudden inundation of the desiccated Mediterranean by Paratethyan waters from eastern Europe (Fig. 3).

Fig. 2 Middle Miocene (16–18 Myr) Palaeogeography.



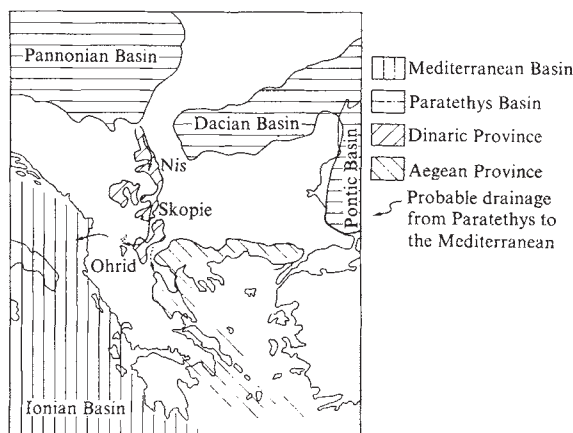


Fig. 3 Late Miocene (5.5 Myr) Palaeogeography.

That such an invasion did take place has been speculated by biogeographers on the basis of the occurrence of relic Paratethys faunas (including freshwater fish, molluscs and birds) in isolated areas of circum-Mediterranean land today³². The evidence from Paratethys also indicated that it suddenly lost much of its waters to the Mediterranean in late Neogene³³. In fact, the drainage reorganisation at this time is believed to have resulted in an evaporative excess of the hydrographic budget of the Black Sea, leading to a partial desiccation of that deep Paratethyan basin³⁴.

The *Lago Mare* may have occasionally extended to the western Mediterranean. The *Cyprideis* fauna is present in land sections in Algeria, Spain, Tuscany, and in Sicily²⁴. A brackish water sediment is present in the Balearic Hole 124 within the evaporite sequence and may be equivalent to the lower part of the *Lago Mare* sequence of the eastern Mediterranean. The very last Messinian sediments there below the Pliocene are, however, restricted marine deposits. It seems that towards the end of the Messinian the marine waters spilled over the western basins before they reached the east. The restricted marine facies of the uppermost Messinian in the Balearic and Tyrrhenian Basins are probably time equivalent in part to the upper *Lago Mare* deposits of the Ionian and Levantine Basins. The latter were inundated after the seawater spilled over Sicilian Channel. The early Pliocene (Trubi) inundation probably did not reach the Red Sea which was separated from the Mediterranean by the Isthmus of Suez. The marine invasion there was dated as occurring some 3.5–4 Myr ago, when a connection to the Indian Ocean was established through the Strait of Bab el Mandeb³⁵.

Pliocene flooding and Plio-Quaternary subsidence

Leg 42A drilling confirmed the Leg 13 drilling results that the first Pliocene sediments above the Messinian are deep and open marine hemipelagic sediments. The Messinian salinity crisis was ended by the Pliocene flooding.

Palaeobathymetric analyses indicated that the Pliocene sediments immediately above the Mio-Pliocene contact are in excess of 1,000 m, or 1,500 m in depth. The drilling evidence proved that deep Mediterranean depressions which had existed before the Messinian salinity crisis were still there at the end of the crisis. Seismic evidence indicates considerable Plio-Quaternary subsidence³⁶. The subsidence is in part induced by the isostatic load of the water flooding the basins⁴. Additional subsidence could be related to cooling of the mantle under the basins, or to continued tectonic activities which may have deepened the Mediterranean back-arc basins²⁶. There have been considerable Plio-Quaternary tectonic activities in the circum-Mediterranean regions. The evaporite basins of Sicily, Calabria,

Apennines, Tellian Atlas, Ionian Islands, Crete, Cyprus, and so on, were uplifted and emerged. Meanwhile continental margins locally (Nile and Rhone deltas, Israeli shelf, and so on) underwent subsidence³⁷. Those activities have modified the post-Messinian palaeogeography. The fact remains, however, that the Mediterranean basins owed their genesis to earlier movements, even though their floor may have been uplifted or subsided somewhat during the last five million years.

Impact of the Mediterranean salinity crisis

The desiccation of the Mediterranean took place 5.5 Myr ago. Such a geologically recent catastrophic event left behind not only a giant evaporite deposit, it also had a great impact on the modern world, on the circum-Mediterranean landscape, on regional and global climates, and on the evolution and distribution of plants and animals.

The lowering of the base-level of erosion led to the deep incision of rivers which drained into the Mediterranean⁴; even the southern Alpine lakes may have been Messinian canyons modified by Pleistocene glacial erosion³⁸. The exposure of Mediterranean bottom promoted an unusually active Late Miocene groundwater movement, which was probably responsible for the initiation of karst topography in Yugoslavia and in other Mediterranean countries. The uneven topography of the Mediterranean Ridge may have been a modified karst-development⁴, anomalous drilling behaviour noted during Leg 42A drilling on the Ridge suggests presence of submarine caverns.

The salinity crisis induced a continued change towards a cooler and more arid climate in the circum-Mediterranean. The Antarctic ice sheet expanded greatly during the Messinian³⁹, and the Arctic ice sheets may have begun to form then⁴⁰.

Biologists are only beginning to explore the implications of the Miocene Mediterranean desiccation. The present Mediterranean fauna is similar to that of the Pliocene, but very different from that of the Miocene, a fact noted by Lyell more than 100 yr ago when he first established the Miocene and Pliocene Epochs. The periodic conversion of an inland sea into dry land also permitted a migration of land animals; numerous examples of exchanges between Africa and Europe during the Messinian have been found^{41,42}. With the sudden Pliocene flooding, animals stranded on the Mediterranean islands would become isolated and develop endemism^{41,42}. Other usual aspects of the present Mediterranean land faunas may also be related to the unusual Messinian event. We might ask, for example, if the unusual habit of the circum-Mediterranean eels to breed in the Mediterranean, rather than in the Sargasso Sea, might be traced back to the time when the gate to the Atlantic was closed. We might also invoke the Messinian *Lago Mare* expansion to explain the similarity of relic freshwater faunas in France, Spain, and North Africa to those of the present Caspian. The impact of the Messinian salinity crisis and the accompanying climatic changes must have greatly influenced the distribution of land plants. The aridity led to temporary or permanent expansion of savanna vegetations. The impact on plant-evolution is subtle, but intriguing. It was suggested that some of the Mediterranean floras (for example *Medicago*) evolved from perennial to annual forms so as to endure longer period of severe drought, and from cross- into self-pollinators because insects could not have followed the new hardy plants into the hot dry environment (K. Lesins, personal communication).

We close this article in a contemplative mood. Biochemical studies suggest that the African ape-human divergence took place 5 or 6 Myr ago during the Messinian^{43,44}, just about the time when what is known to be the earliest hominid lived in Kenya⁴⁵. Is the timing

purely coincidental, or did the great expansion of the East Africa savannas at the time of the Mediterranean salinity crisis promote hominid evolution? Is our very being today traced back to that unusual event?

Received 27 January; accepted 1 April 1977.

- 1 Hsü, K. J., Ryan, W. B. F. & Cita, M. B. *Nature* **242**, 240 (1973).
- 2 Hsü, K. J. *Earth Sci. Rev.* **8**, 371 (1972).
- 3 Sylvester-Bradley, P. C. *Geol. Mag.* **110**, 73 (1973).
- 4 Hsü, K. J., Cita, M. B. & Ryan, W. B. F. *I.C.R. DSDP (Initial Reports of the Deep Sea Drilling Project, U.S. Government Printing Office, Washington DC) 13*, 1023 (1972).
- 5 Nesteroff, W. D. *ICR DSDP 13*, 673 (1972).
- 6 Wezel, F. C. *Giornale di Geologia* **39**, 447 (1974).
- 7 Drooger, C. W. in *Messinian Events in the Mediterranean* (ed. Drooger, C. W.), 263 (North-Holland, Amsterdam, 1973).
- 8 Argand, E. *XIIIe Congr. geol. intern. Bruxelles*, CR 171 (1924).
- 9 Selli, R. & Fabbri, M. R. C. *Accad. Lincei* **8**, 104 (1971).
- 10 Neev, D., Almogor, G., Arad, A., Ginsburg, A. & Hall, J. K. *Geol. Surv. Israel, Bull.* **68**, 1 (1976).
- 11 Smith, A. G. *Geol. Soc. Am. Bull.* **82**, 2039 (1971).
- 12 Dewey, J. F., Pittman, W. C., Ryan, W. B. F. & Bonin, J. *Geol. Soc. Am. Bull.* **84**, 3137 (1973).
- 13 Biju-Duval, B., Dercourt, J. & Le Pichon, X. *Recherche* **7**, 811 (1976).
- 14 Hsü, K. J. & Bernoulli, D. *ICR DSDP 42A* (in the press).
- 15 Wright, R. *ICR DSDP 42A*, (in the press).
- 16 Benson, R. H. *ICR DSDP 42A*, (in the press).
- 17 Bernoulli, D., Garrison, R. E. & McKenzie, J. A. *ICR DSDP 42A* (in the press).
- 18 Suess, E. *Nat. Sci.* **2**, 180 (1893).
- 19 Hallam, T. in *Palaeontological Ass. spec. Publ.* **12** (ed. Hughes, N. F.) (1973).

- 20 Adams, C. G. in *Atlas of Paleobiogeography* (ed. Hallam, A.) 453 (Elsevier, Amsterdam, 1973).
- 21 Savage, R. J. G. *Systematics Ass. Publ.* **7**, 247 (1967).
- 22 Rögl, F., Steiniger, F. & Müller, C. *ICR DSDP 42A* (in the press).
- 23 Gignoux, M. *Geologie Stratigraphique*, 4th edn (Masson & Cie, Paris, 1950).
- 24 Cita, M. B., Wright, R., Ryan, W. B. F., & Longinelli, A. *ICR DSDP 42A* (in the press).
- 25 Benda, L. in *Messinian Events in the Mediterranean* (ed. Drooger, C. W.), 256 (North-Holland, Amsterdam, 1973).
- 26 Montadert, L. C., Letouzey, J. & Mauffret, A. *ICR DSDP 42A* (in the press).
- 27 Decima, A. & Wezel, F. C. *ICR DSDP 13*, 1234 (1972).
- 28 Decima, A. *Proc. Messinian Seminar, Erice, Sicily* (1975).
- 29 Schreiber, B. C., Friedman, G. M., Decima, A. & Schreiber, E. *Sedimentology* **23** (1976).
- 30 Garrison, R. E. *et al. ICR DSDP 42A* (in the press).
- 31 McKenzie, J. A. & Ricchiuto, T. E. *ICR DSDP 42A* (in the press).
- 32 Stankovic, S. *The Balkan Lake Ohrid and its Living World Monographiae Biologicae* **9** (W. Junk, The Hague, 1960).
- 33 Jiricek, R. *VIIth Congr. Med Neogene Stratigraphy, Bratislava*, 33 (1975).
- 34 Hsü, K. J. *ICR DSDP 42B* (in the press).
- 35 Hsü, K. J., Stoffers, P. & Ross, D. A. *Abstr. 25th Int. Geol. Congr. Sydney*, 894 (1976).
- 36 Morelli, C. *Bull. de l'étude en commun de la Méditerranée* **7**, 29 (1975).
- 37 Ryan, W. B. F. *Sedimentology* **23**, 791 (1977).
- 38 Finckh, P. *Messinian Seminar 2* (Gargnano, Italy, 1976).
- 39 Hayes, D. E. *et al. ICR DSDP 28* (1975).
- 40 Van Hinte, J. *ICR DSDP 38* (1976).
- 41 Hsü, K. J. *Naturwissenschaften* **61**, 137 (1974).
- 42 Azzaroli, A. & Guazzoni, G. *Messinian Seminar 2*, 60 (1976).
- 43 Sarich, V. M. & Wilson, A. C. *Science* **158**, 1200 (1967).
- 44 Goodman, M. *et al. Aeromedical Res. Lab. ARL-TR-69-10* (Holloman A. F. B., 1969).
- 45 Ardrey, R. *The Hunting Hypothesis* 47 (Collins, London, 1976).

Catastrophic chemical events in the history of the ocean

William T. Holser

Max-Planck-Institut für Chemie, D-6500 Mainz, Germany (BRD)

Department of Geology, University of Oregon, Eugene, Oregon 97403

Catastrophic chemical events are characterised by sharp rises in $\delta^{34}\text{S}$ in the surface of the whole world ocean, and by greater overshoots locally. Three events are recognised and named for the formation in which they are most sharply displayed. The sharpness of the rise in $\delta^{34}\text{S}$ suggests that the sulphide deposition necessary to explain it must have been accumulating residual high- $\delta^{34}\text{S}$ seawater for some tens of millions of years out of contact with the surface ocean. A modified geological model is presented: brine generated by evaporite deposition is stored in deeps of a mediterranean basin; underneath the brine, pyrite precipitation builds a store of brine heavy in $\delta^{34}\text{S}_{\text{SO}_4}$, whose corresponding buildup of $\delta^{38}\text{O}_{\text{SO}_4}$ may balance the decrease of $\delta^{38}\text{O}_{\text{SO}_4}$ from evaporite deposition. Catastrophic mixing of the brine and the surface ocean, initiated by destruction of the storing basin, is the source of the sharp rise in the sulphur isotope age curve detected world-wide in evaporites. These events have important implications not only for modelling of the chemical history of the ocean, atmosphere, and sediments, but also for the explanation of faunal crises, and the many aspects of geology that depend on the composition and circulation of the oceans and their peripheral basins.

It has been known for some time that the sulphur isotope ratio in the world ocean surface has undergone major excursions during the period (Proterozoic–Phanerozoic) that could be documented from evaporite sulphate samples^{1–3}. The resulting sulphur isotope age curve has recently been updated with many new analyses of sulphur isotopes^{4,5}, supplemented by measurements of oxygen isotopes from some of the same sulphates^{5,6}.

The interpretations of these isotope age curves have focused mainly on explaining the trends of $\delta^{34}\text{S}$ as a result of widespread variations in biological reduction of sulphur into shales; on subsequent oxidative erosion into the sea; and with possible coupling of the carbon cycle to maintain atmospheric oxygen (refs 3, 5, 7–12). My purpose here is to discuss some more dramatic but less well-documented features of the isotope age curves, and to discuss whether they record catastrophic chemical events that are specific in time and place.

Upward jumps in $\delta^{34}\text{S}$

Fig. 1 shows as a solid line the mean best estimate for $\delta^{34}\text{S}$ in sulphate evaporite minerals in equilibrium with the surface of the world ocean^{4,5}. In constructing this curve greater weight was attributed to values whose world-wide distribution bore out their origin in the ocean surface as a whole, and less weight to results that were found in a narrow range of time and place. But it is worth noting that those of the latter deviations that are not so widespread may have considerable significance.

Marks on Fig. 1 highlight three rises in $\delta^{34}\text{S}$; and a solid line in Fig. 2 repeats these parts of the curve on an enlarged scale. These rises are so large and take place over such a short time, that I have labelled them chemical events by analogy with the nomenclature of magnetic reversals. Table 1 lists some characteristics of these three events. There are differences that may be significant, but some common features are: (1) The mean curve established by world-wide evaporite sampling rises sharply. (2) The rise is highly accentuated by a positive overshoot, in one formation in one basin, which gives its name to the event. The overshoot peak is a step function within the limits of stratigraphic control. (3) It is difficult to fix the absolute time interval within which the event is bracketed. Each seems to have taken place within one stratigraphic stage, probably much less than 5 Myr. (The times given in Table 1 are only scaled by inter-

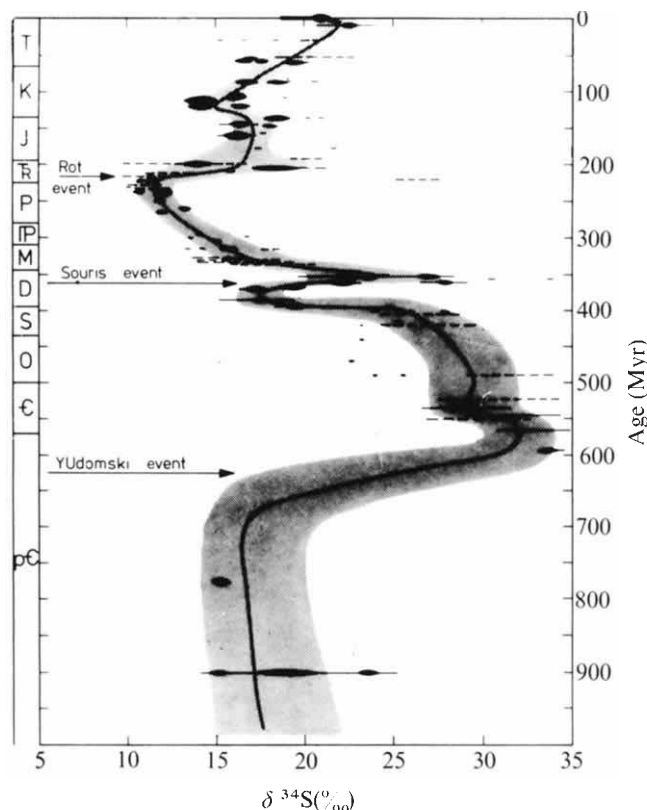


Fig. 1 Summary sulphur isotope age curve for marine sulphate, from Claypool, *et al.*^{4,5}, who give detailed plots and lists of analyses. Data are shown here by areas or lines that indicate qualitatively the number of analyses, plotted at their most probable age. The heavy line is the best estimate for $\delta^{34}\text{S}$ in equilibrium with the world surface ocean of that time; the shaded area is an estimate of the uncertainty of that determination. The named catastrophic chemical events are sharp rises in the best-estimate curve, accompanied by very high values ('overshoots') found only locally.

polation from radioactive dates that are for the most part separated by a whole geological period¹³.) (4) Each of the events is followed by a long period of mean oceanic $\delta^{34}\text{S}$ that is higher than the pre-event level, and of course lower than the overshoot peak. This post-event level is either constant (Yudomski and Röt Events) or relatively slowly decreasing (Souris Event) over a substantial period of time. (5) The mean curve for $\delta^{18}\text{O}_{\text{SO}_4}$ in

marine sulphates⁵ at equivalent times displays either no discernible rise (Yudomski and Souris Events) or a relatively small one (Röt Event).

Nature of the chemical event

Models for interpretation of the sulphur isotope age curve explain general rises as indicative of marine biological precipitation of sulphide predominant over weathering of sulphide, and falls as oxidative sulphide weathering (refs 4, 5, 8, 11, 12), although the models differ widely in assumed constants, residence times, and couplings. Processes of a similar nature must also explain the sharp events.

Detailed consideration of the isotope geochemistry of oxygen has led Claypool *et al.*^{4,5} to conclude that when $\delta^{34}\text{S}$ rises a corresponding rise in $\delta^{18}\text{O}_{\text{SO}_4}$ can only be prevented if the sulphide deposition is accompanied by extensive evaporite deposition. This is possible because with oxygen, much more than with sulphur, the isotope effect of evaporite deposition tends to offset the effect of sulphide deposition. Consequently, the chemical events seem to have involved major and contemporaneous deposition of sulphide into anaerobic muds and of sulphate into evaporities.

But these rises in the sulphur curve, and especially the local overshoots, are so precipitous that an extremely high rate of sulphide precipitation would be required to change the whole world ocean so much in so short a time. One can first of all see qualitatively that if the residence time of sulphur in the ocean (with cyclic sea salt subtracted) is about 20 Myr, as at the present time³, then it will be very difficult to generate changes of 5–15‰ in $\delta^{34}\text{S}$ within 5 Myr, and still more so in 1 Myr. Nielsen² calculated that the rate of change of $\delta^{34}\text{S}$ probably would not exceed 0.3‰ per Myr. In making this calculation, he estimated the present rate of sulphur erosion ($\approx$ deposition) of 15 Mt yr⁻¹, but the maximum rate would have meant the complete suspension of erosion to get the calculated rate of rise from sulphur deposition. This seems unlikely, especially as present rates of erosion may be higher than usual¹⁴. Nielsen had also to use the current value of the sulphur content of the ocean. It is true, however, that if the ocean were smaller, the same rate of sulphide deposition would raise $\delta^{34}\text{S}$ correspondingly faster. Holland¹¹ also assumed the present sulphur content of the ocean, as well as the present rate of sulphur input by rivers (uncorrected for cyclic sea salt and anthropogenic sulphur). His values of rises in available oxygen for the intervals that span my chemical events can be converted to amounts (of sulphur deposited) that are correspondingly large,

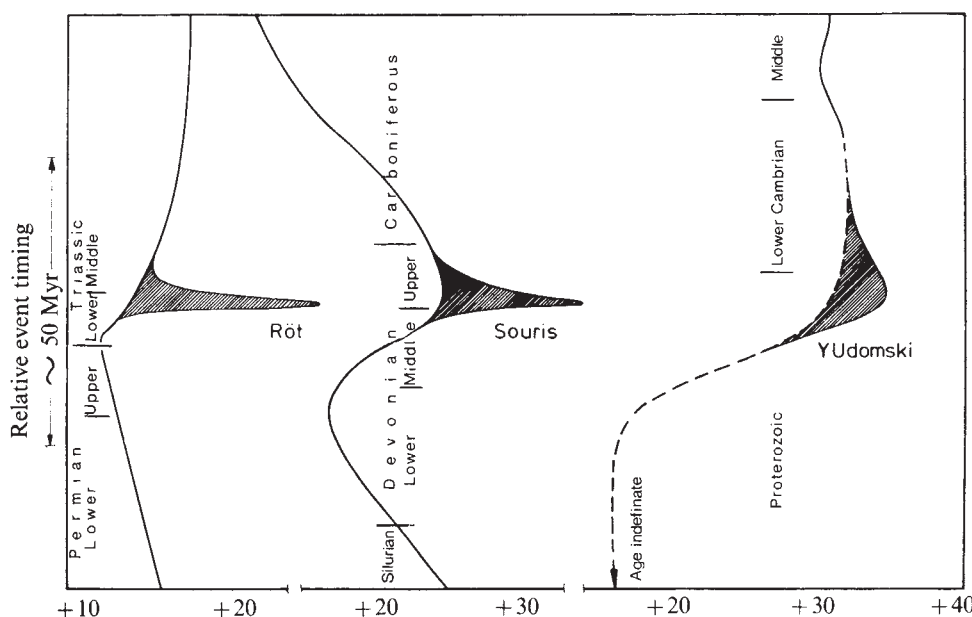


Fig. 2 The chemical events labelled in Fig. 1 are shown here schematically on greatly enlarged scale. Solid line: best estimate for $\delta^{34}\text{S}$ of sulphate mineral in equilibrium with world surface ocean; shaded area: local high overshoot. The timing is based approximately on stratigraphic stages, so that the absolute time scale is very approximate.

Table 1 Catastrophic chemical events

Suggested name Stratigraphic stage	YUdomski Event Pre-Aldan (Latest Proterozoic)	Souris Event Frasnian (Lower Upper Devonian)	Röt Event Scythian (Upper Lower Triassic)
Approximate age (Myr)	575	355	215
Pre-event level $\delta^{34}\text{S}$			
Stratigraphic stage	Late Proterozoic	Eifelian (Lower Middle Devonian)	Mittlerer Buntsandstein (M. L. Triassic)
Approximate age (Myr)	635–850	365	220
Mean $\delta^{34}\text{S}$, ‰	+15	+18	+13
Earliest peak in $\delta^{34}\text{S}$ Formation	YUdomski Horizon	Souris River and others	Röt
Location	Eastern Siberian Platform	Saskatchewan	Netherlands to DDR
Other localities	Saline Series, Pakistan	—	Moenkopi of Utah
Maximum $\delta^{34}\text{S}$	Hormuz Series, Iran		
Maximum excursion in $\delta^{34}\text{S}$	+35 +20	+34 +16	+27 +14
Post-event level $\delta^{34}\text{S}$			
Stratigraphic stage	Lower to Middle Cambrian	Famenian (Upper Upper Devonian)	Muschelkalk (Middle Triassic)
Mean level	+30	+23	+19
Rise of mean $\delta^{34}\text{S}$	+15	+5	+6
Rise of mean $\delta^{18}\text{O}$	0?	+0.5	+2.5

and when compressed into an actual rise time even as long as 5 Myr, the rate of net sulphur deposition figures out to about 150 Mt yr⁻¹. Using the simultaneous solution of mass balances for both sulphur and oxygen isotopes I have also calculated similarly extreme rates of sulphide deposition. These calculated rates all seem unlikely, and suggest that the deposition of sulphide that was responsible for the rise in the age curve was accumulated over a long period of time, but was read into the evaporite record in a very short time.

The first evidence of the event is also the strongest peak, and in at least the YUdomski and Souris Events is found in only one basin. This suggests a chemical overshoot, possibly in the very area where the event was generated. The post-event level is seen on the whole ocean's surface, and the eventual decline corresponds to a more uniform decay through the weathering process.

A geological model

To explain the 'Permo-Triassic faunal crisis' (which is coincident with my Röt Event) Fischer¹⁵ suggested that brine was generated on shelves from evaporite or near-evaporite conditions, and then slid down as a density current to accumulate in the ocean depths. This mechanism generates a stratified ocean that is at least temporarily stable, with brine below and a brackish surface layer. The characteristics of the isotopic events suggest the following modifications of Fischer's proposal.

Fischer required brine that was to some undefined degree dense enough to stably resist mixing; he allowed but did not require that evaporation proceed as far as evaporite precipitation. The oxygen isotope data indicate that evaporites of at least the calcium sulphate facies were generated in large amounts. The resulting brine, of density 1.10–1.20 × 10³ kg m⁻³ has therefore all the more likely to be hydraulically stable in the bottom of a basin. Other proposals^{16,17} have been based on evaporite deposition in the halite facies, and that is not precluded here.

Fischer suggests a deep-sea depression as the storage site for brine. That is not necessary, and it may not be correct. The bottom of any basin will do for brine storage, so long as it is large enough to hold the amount of brine that must be sequestered to account for the change of $\delta^{34}\text{S}$. A rift graben or other mediterranean has the advantage that it is more susceptible to the quick destruction suggested by the fast rise of the $\delta^{34}\text{S}$ curve, which represents chemical mixing of the deep brine and the surface layer.

The suggestion of a large basin in which evaporite deposition (as well as sulphide deposition) is important will probably remind the reader of the desiccating sub-sea level basin^{18,19}, which

enjoys inflow from the ocean without reflux. One problem with that arrangement is that in conditions approaching desiccation, the required mixing of the brine is inhibited. Valiashko²⁰ has pointed out that even at the beginning of the potash evaporite facies, the volume of remaining brine may be accommodated in the porosity of the previously deposited salts. But the basin could be sub-sea level, and non-refluxing, so long as it was not desiccating.

It therefore seems most likely that the site for brine accumulation is neither a deep-sea basin, nor a sub-sea level desiccating basin, but rather a large mediterranean isolated from the ocean, especially with regard to its depths.

A particular requirement for the chemical event is that evaporite deposition be accompanied by sulphide deposition, within the same basin complex if not actually the same basin. Although many evaporite sequences are grey and evidently associated with reducing conditions, the amount of sulphide is usually small. So it may be that the major part of the sulphide deposition occurs under brine accumulated nearby. Some present saline depths support bacterial reduction of the large proportion of sulphate that they still retain, while others are sterile. The differences are not understood^{21–23}.

Sulphide deposition requires not only the generation of sulphide by sulphur-reducing bacteria, but also a sufficient supply of iron (ferrous or ferric) to fix the sulphide in the sediment. Not much sulphide can be retained in solution in the brine, and even this would be ineffective for the chemical event if it were simply remixed with the ocean. Although sulphide deposition in ordinary bottom muds is usually supposed to get its iron from the sediments themselves (probably from hematite or goethite coating sand grains)²⁴, the amount required for building the large reservoir of fixed sulphide suggests that supplies could well be augmented from a more plentiful source such as submarine volcanism^{25,26}.

According to Fischer¹⁵ the fossil record suggests that during the Triassic the surface ocean returned to normal salinities. The sulphur isotope record also suggests this—but the sharp onset indicates a more catastrophic mixing of brine and surface ocean. At that time, in addition to the restoration of normal salinity described by Fischer, we may have a catastrophic inflow in various places of waters rich in salts, organic matter, sulphide ions, nutrients, oxygen demand, and $\delta^{34}\text{S}$. The local nature of the overshoot in $\delta^{34}\text{S}$ is further evidence for the catastrophic nature of the mixing. The location of such an overshoot suggests but does not necessarily prove that the brine was stored in a basin nearby.

In accordance with current custom much of the above can be stated in terms of the theory of plate tectonics. The rifting Atlantic is prominently associated with evaporites (refs 19, 27

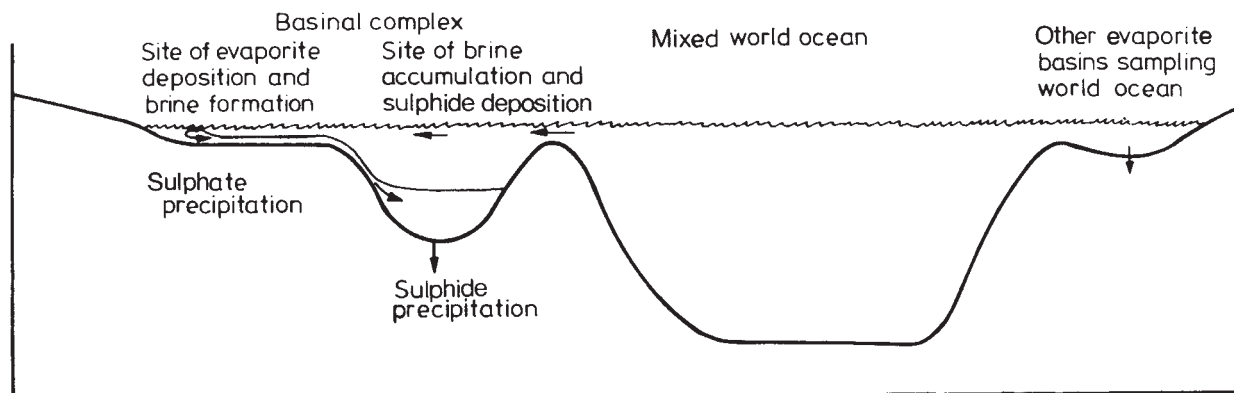


Fig. 3 Geological model to explain the chemical events, somewhat modified from Fischer¹⁵. A basin or basin complex precipitates sulphate, and generates and stores brine, under which reduced sulphide is precipitated. The evaporites of the world (both left and right) sample only the surface ocean, out of contact with sulphate residues of the sulphide reduction, until its accumulated effect is catastrophically mixed with the surface waters at the time the basin complex is destroyed.

and 28); and Kinsman (refs 17, 29 and 30) has generalised and explained this association in the following way: rifting leads not only to initial rift valleys like the Red Sea, but also continuously generates subsiding continental margins, as well as transverse tensional grabens. These are all potential sites of restricted circulation and evaporite deposition along the trailing continental edge. Isolation may be aided by island-forming volcanism in the rift. Kinsman¹⁷ also pointed out the fast timescale of evaporite generation on specific sections of a continental margin—his time limit of 10 Myr is compatible with the chemical event reported here. I need only add to this description that brine sliding down and accumulating in the rift basin will help reduction of sulphide, and volcanic activity or associated thermal waters may supply iron and other heavy metals to fix the sulphide in giant deposits^{25,26}. Eventual full separation of the continents mixes brine and surface ocean. This scenario is a possible one, but other tectonic situations may also generate the required structures and geography.

History of a chemical catastrophe

The story line is indicated in Fig. 4. At time *A* we find the sulphur isotope age curve slowly declining as a result of erosion of predominantly sulphide material into a mixed ocean. This erosion exceeds the sulphide deposition. At time *B*, dense residual brines from evaporite deposition begin to accumulate in the deep of a large nearby basin. Reduction to sulphide continues and $\delta^{34}\text{S}$ in the brine gradually begins to reverse and then increase, while at the same time its mass continues to grow as a larger and larger proportion of the ocean's sulphate is processed through the sulphate-sulphide production system. Meanwhile, the surface layer becomes more brackish, and its $\delta^{34}\text{S}$ continues to fall under the influence of the continuing erosion of older sedimentary sulphide. At point *C* the accumulated brine with high $\delta^{34}\text{S}$ begins to leak or mix slightly with the surface body of the ocean, slowing and even reversing the downward trend of $\delta^{34}\text{S}$ recorded in evaporites everywhere. At point *D* the climax of the event is reached as the brine is released, perhaps through tectonic destruction of the basin. Local appearance of the high $\delta^{34}\text{S}$ may be sampled by evaporites as an isolated record of $\delta^{34}\text{S}$ overshoot. Mixing of brine and surface water leads to an intermediate level of $\delta^{34}\text{S}$ in the subsequent evaporite sampling of the now mixed ocean. As erosion assumes greater importance again, at point *E*, $\delta^{34}\text{S}$ begins to decrease. This may continue until the required combination of evaporite production and basin geometry again induces brine accumulation.

Point *A* does not leave a record that I know how to recognise, so I don't know how long before the event brine segregation and accumulation began. It is conceivable that all the depressed sections of the $\delta^{34}\text{S}$ curve represent segregation and that therefore erosion never really takes over, but only becomes more evident when its depositional counterpart is hidden in a deep.

I realise that the explanation proposed for the chemical events is only a skeleton, and I exhibit it here so that others may join me in either giving it flesh or burying it. Whether it is viable or not the events are real and demand another explanation if not this one.

Geological model

Each of the events displays some individual character in the isotope age curves—these will now be described. And to follow up on the conjecture that the location of brine storage is flagged by the overshoot, the palaeogeography will be examined for conditions favourable to brine accumulation and sulphide reduction.

YUdomski Event. This event occurs just below the boundary of the Proterozoic and the Cambrian, between the Shaler Group of the Arctic Islands that can only be bracketed at 630–850 Myr (ref. 31), and the YUdomski Horizon of the Siberian Platform that is said to be dated at 600 ± 10 Myr (ref. 32). So the abruptness of the rise shown in Figs 1 and 2 is drawn by analogy to the later two events. Within the vague limits of the available data, $\delta^{18}\text{O}$ is unchanged throughout the whole event⁵. The level $\delta^{34}\text{S} \approx 17\text{‰}$ in the Proterozoic pre-event stage is puzzling. It could be an artefact of an imperfect record. Perhaps brine was accumulating all that time, leading to the climax of the chemical event at the Cambrian boundary. But in that case one would have expected the surface ocean as sampled by the evaporites to have shown a declining curve due to erosional input.

The earliest record of this event is an overshoot to 33‰ in our new analyses of the YUdomski or Motskaya Suite, which crops out around the southern edge of the Irkutsk Amphitheatre in Siberia. This horizon lies comfortably below the Cambrian³³, which especially in its middle stage developed into one of the world's largest evaporite basins²⁴. There are earlier evaporites, as yet unanalysed for isotopes, in Siberia that precede the YUdomski³². All of these Siberian basins opened in the direction of the present Arctic, and that could have been the locale for brine accumulation, but its palaeogeography and tectonics are unknown to me.

A possible alternative site for the main event is suggested by one analysis of high $\delta^{34}\text{S}$ in the Cambrian of the Salt Range of Pakistan⁶; this horizon evidently overlies the extensive Saline Series of possible Late Precambrian age³⁵, which may also be correlative with the very extensive Hormuz Series evaporites that extend across southern Iran^{36,37}. This has been confirmed by unpublished high values of $\delta^{34}\text{S}$ obtained by Heimo Nielson (personal communication) from several samples of the Hormuz Series.

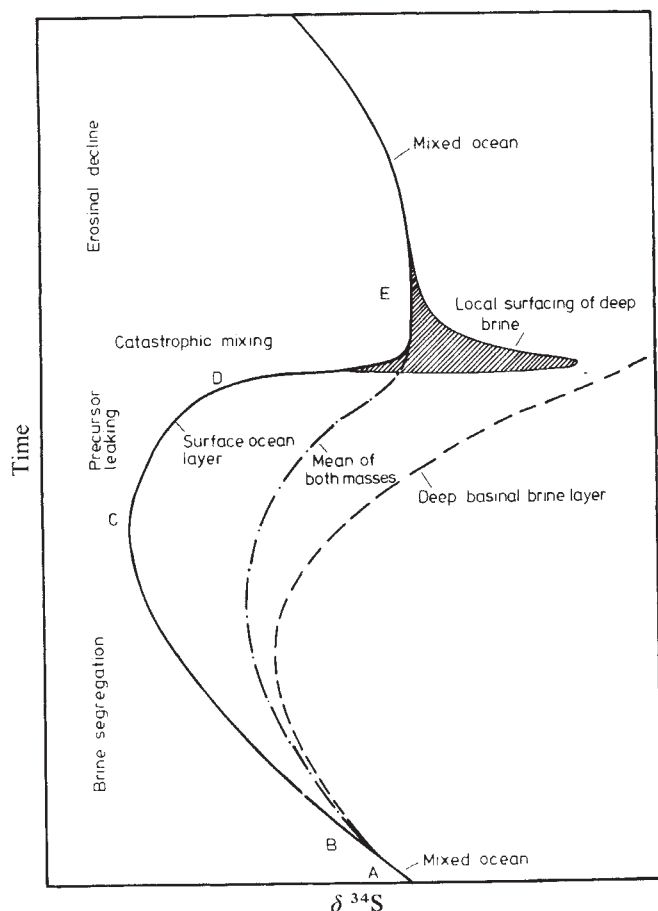
Souris Event. A special character of this event is a well-documented gradual rise in worldwide $\delta^{34}\text{S}$ during the Middle Devonian, that precedes the sharper rise to the peak in the Famennian Stage^{6,7}. In terms of the model, this represents a more gradual leak or mixing from the stored brine into the ocean.

A strong overshoot is displayed in the Souris River Formation and related beds near the top of a giant sequence of evaporites that accumulated in the Elk Point Basin of Western Canada, beginning in the Middle Devonian. The basin was recharged from the north, across the strongly developed Presquile Reef in the North-west Territories. Beyond the reef black marine shales were extensively deposited³⁹, and this area may have been the place of brine accumulation and sulphide reduction. It lies at the edge of the Eurasian basin, whose palaeogeography and tectonics are still controversial^{39,40}.

Röt Event. This event occurs sometime between well-established low levels of $\delta^{34}\text{S} = 11\text{‰}$ in the latest Permian, and the moderate levels of the Middle Triassic and later Mesozoic stages. Although in many areas stratigraphic subdivision of the Lower Triassic has not been successful, analyses from the well divided Buntsandstein of East Germany⁴¹ and West Germany (unpublished data by Dietrich Rambow and Heimo Nielsen) show that low values of the Permian continue through the Lower and Middle part of the Lower Triassic.

The Buntsandstein exhibits a most dramatic overshoot, up to $\delta^{34}\text{S} = +27\text{‰}$, in the Röt (Upper Lower Triassic), whose evaporite facies extend from Poland across Germany (East and West) into England^{42,43} and touch Greenland⁴⁴. The main

Fig. 4 Schematic representation of sulphur isotope effects during the course of a chemical event. At the earliest time, *A*, $\delta^{34}\text{S}$ is slowly decreasing as erosion of sulphide exceeds deposition of sulphide. At *B* brine starts to accumulate in a basin complex as a result of evaporite deposition (Fig. 3), and biological reduction under the brine begins to slow and then reverses the decline of $\delta^{34}\text{S}$ in the residual sulphate in the brine, but out of contact with the surface ocean which continues to decline in $\delta^{34}\text{S}$ (as sampled by worldwide evaporites). At *C* the brine begins to mix somewhat with the surface ocean, and at *D* mixing becomes catastrophic, with local overshoot due to surfacing of deep brine. At *E* a level for the now mixed ocean is a weighted mean of the previous two reservoirs of sulphate; the course of this fictive mean isotope composition during the event is shown by the middle line.



development of Röt evaporites coincides with much of the thickest Zechstein evaporites. If, as seems plausible^{42,43}, the Röt and Zechstein had a common entrance to the north ('Skandik Sea'), this might furnish a suitable site for brine accumulation; although an alternative opinion is that the Röt was open to the Tethys Sea to the south-east⁴⁵.

A few analyses⁵ suggest a possible alternative site for this event, in the Lower Triassic areas of the Western United States.

Note that all three events have possible connections with brine accumulations in the region of the Palaeozoic geography that lies in the present Arctic.

Comparison with other theories

Thode and Monster¹ proposed that certain basins would have high values of $\delta^{34}\text{S}$, due to greater amounts of sulphur reduction, and they therefore assumed as a value for the world ocean the lowest $\delta^{34}\text{S}$ in any basin at a given time. In the present instance reduction in a basin is also proposed, on an even larger scale, but its residues remain out of contact with the world ocean surface until the catastrophic event of mixing.

Pilot *et al.*⁴¹ dealt specifically with the Röt event: They proposed that an accumulation of sulphide reduction in the Tethys Sea had been going on since the Carboniferous, and that the Röt evaporites were drawn from that sea, in contrast with the Zechstein which was drawn from the northern sea. For my model it is not sufficient to isolate Tethys and deposit sulphide there so that the Röt can tap it with a high $\delta^{34}\text{S}$ evaporite. The sulphide-depositing basin also has to derive a large amount of sulphate from the world ocean, while at the same time holding back its high $\delta^{34}\text{S}$ until a critical moment. I accomplish this with the evaporite-generated brine layer. This could have happened in Tethys, but for the reasons described above I think the northern sea is more likely.

Other speculations concerning the history of the ocean, while not specifically directed towards the interpretation of isotope age curves, are related to major chemical events. Buerlen¹⁶ proposed that the faunal crisis at the Permian-Triassic boundary might be connected with a decreasing salinity of seawater due to the extensive precipitation of evaporites during the Permian. As indicated above, Fischer¹⁵ had a similar explanation for this palaeontological problem, but made the surface ocean brackish by the accumulation of deep brines rather than necessarily by deposition of salt. His paper was the real ancestor of this one, but its significance was not appreciated until the isotope data had been accumulated in sufficient detail. Kinsman¹⁷ showed how the kind of events proposed by Buerlen (although he did not refer to either Fischer or Buerlen) may have been very strong during the early phases of a continental rifting. None of these proposals connected the generation of brackish surface waters with sulphide reduction, which is the main point of the new proposal. More recently, Degens and Stoffers⁴⁶ proposed that density stratification, of unspecified origin, may have been a common phenomenon in earlier seas, on the model of the present Black Sea. Reducing conditions in the brine and the sediment, and accumulation of organic material, are important aspects of the model of Degens and Stoffers. What I am adding to their model is an emphasis on sulphide deposition in the sub-brine sediment, a suddenness of re-mixing with the surface ocean, and a method of specifically detecting such situations in the geological record by the interpretation of the isotope age curves.

Catastrophic chemical events—implications for future work

(1) Modelling of ocean history and the calculations of oxygen demand (positive or negative) associated with sulphide-sulphate transfers will be more complicated than it has been^{4,11,12} under the assumption of a mixed ocean. In principle it will be possible to make new calculations with the sea sulphate divided between two reservoirs, surface and brine. Even without such calculations, one can say qualitatively that the overall decrease of $\delta^{34}\text{S}$ in the pre-event stage will be less

than that for the surface ocean alone, so that the maximum oxygen demand associated with a $\delta^{34}\text{S}$ minimum will be less than would have been calculated assuming a mixed ocean.

(2) The dominant sulphide reduction during the pre-event stage will be under the brine reservoir. Therefore during the deep minimum in the $\delta^{34}\text{S}$ evaporite curve, a corresponding $\delta^{34}\text{S}$ curve for these particular sulphides need not show such a deep minimum or, indeed, any minimum at all. Whether sulphides of the briny deep are mainly in black shales, as seems most likely, or also in large stratiform sulphide deposits, remains to be determined. The limited sulphur isotope data presently available for both styles of deposition have been reviewed by Schwarcz and Burnie⁴⁸, but these represent only a handful of occurrences.

(3) The combination of evaporite deposition and brine formation decreases surface salinity (in all its components) of the ocean during the pre-event phase. The surface ocean should also show a corresponding increase of δD and $\delta^{18}\text{O}_{\text{H}_2\text{O}}$, which should be looked for in fossils, limestones, and cherts, but on a finer time scale than previously tried^{15,48}.

(4) The decrease of sulphate in the surface ocean may allow the buildup, from erosional input, of elements such as barium and strontium; they may be precipitated during the subsequent mixing. Some sedimentary barite deposits^{49,50} coincide exactly with the Souris event; and peaks of strontium in shales are found near the YUdomski and Röt Events⁵¹. The age curve for $^{87}\text{Sr}/^{86}\text{Sr}$ shows a number of sharp rises⁵²⁻⁵⁴, and each of the chemical events postulated here has one of these jumps in $^{87}\text{Sr}/^{86}\text{Sr}$ associated with it: YUdomski Event—from 0.7075 in the Late Proterozoic (570 to 850 Myr?) to 0.7095 in the Lower Cambrian; Souris Event—from 0.7080 in the Givetian and Frasnian to 0.7093 in the Famennian; and the Röt Event—0.7073 in the Kazanian and Tartarian to 0.7083 in the Early Triassic. Veizer and Compston⁵³ noted that the fine structure of the strontium isotope curve might "... indicate that the fluctuations in Sr isotopic composition of seawater could develop within several million years." But the difficulty here is similar to sulphur—a residence time in a mixed ocean that is much longer than that.

(5) The possible effects of these various chemical changes on biological processes, ecology, and evolution may be far-reaching. Some of these have already been discussed in earlier models: pre-event increase in oxygen demand¹¹ (but less than previously assumed¹²), and decrease of surface salinity¹⁵⁻¹⁷. The new model would generate the added stress of a quick return to more or less normal salinity. The remixing of anoxic brines into the ocean may have profound effects, particularly in local regions of overshoot. Nutrients may be removed from effective participation in the biological cycle—temporarily with all seawater constituents, or by sedimentation under the brine. In the post-event stage re-mixing restores to the productive surface waters those nutrients still in solution, with consequent effects on organic productivity and possibly increased deposition of organic carbon and decrease of atmospheric $p\text{CO}_2$. A post-event increase in phosphate in surface waters may account for the proliferation of apatite-secreting metazoa in the Cambrian⁵⁵.

I thank my colleagues G. Claypool, I. Kaplan, H. Sakai, and I. Zak for many discussions in our quest to find the reasons for the isotope age curves. I am grateful for an appointment as Senior Scientist under the programme of the Alexander von Humboldt Stiftung, and for the hospitality of C. Junge and M. Schidlowski at the Max-Planck-Institut für Chemie, Mainz, Germany, where this paper was written. I thank H. Nielsen for stimulating discussion and access to unpublished data.

Received 3 February; accepted 29 March 1977.

- 1 Thode, H. G. & Monster, J. *Am. Ass. petrol. Geol. Mem.* **4**, 367-377 (1965).
- 2 Nielsen, H. *Geol. Rundsch.* **55**, 160-172 (1965).
- 3 Holser, W. T. & I. R. Kaplan *Chem. Geol.* **1**, 93-135 (1966).
- 4 Claypool, G. E., Holser, W. T., Kaplan, I. R., Sakai, H. & Zak, I. *Geol. Soc. Am. Programs Abstr.*, **4**, 473 (1972).
- 5 Claypool, G. E., Holser, W. T., Kaplan, I. R., Sakai, H. & Zak, I. (Unpublished).
- 6 Sakai, H. *Earth planet. Sci. Lett.* **15**, 201-205 (1972).
- 7 Holser, W. T. & Kaplan, I. R. *Geol. Soc. Am. Spec. Pap.* **101**, 97 (1968).
- 8 Rees, C. E. *Earth planet. Sci. Lett.* **7**, 366-370 (1970).
- 9 Nielsen, H. *UNESCO Earth Sci. 7*, 91-102 (1972).
- 10 Nielsen, H. in *Mezhdunar. Geokhim. Kong. (Dokl.)*, **1st**, 1971 (ed. Vinogradov, A. P.) **4**, 1, 127-140 (1973).
- 11 Holland, H. D. *Geochim. Cosmochim. Acta* **37**, 2605-2616 (1973).
- 12 Schidlowski, M., Junge, C. E. & Pietrek, H. *J. geophys. Res.* (in press).
- 13 Harland, W. B., Smith, A. G. & Wilcock, B. (eds) *Q. Jl. geol. Soc. Lond.* **120S** (1964).
- 14 Garrels, R. M. & Mackenzie, F. T. *Evolution of Sedimentary Rocks* (Norton, New York, 1971).
- 15 Fischer, A. G. in *Problems in Palaeoclimatology* (ed. Nairn, A. E. M.) 566-574 (Discussion: p. 575-577) (Interscience, London, 1964).
- 16 Buerlen, K. Z. *Deutsch. Geol. Gesell.* **108**, 88-99 (1956).
- 17 Kinsman, D. J. J. *Nature* **255**, 375-378 (1975).
- 18 Hsu, K. H. *Earth Sci. Rev.* **8**, 371-396 (1973).
- 19 Burke, K. *Geology* **3**, 613-616 (1975).
- 20 Valiaschko, M. G. *Freiberger Forschungs. A123*, 197-233 (1958).
- 21 Trüper, H. G. in *Recent Heavy Metal Deposits in the Red Sea* (eds Degens, E. T. & Ross, D. A.), 263-271 (Springer-Verlag, Berlin, 1969).
- 22 Watson, S. W. & Waterbury, J. B. in *Recent Heavy Metal Deposits in the Red Sea* (eds Degens, E. T. & Ross, D. A.), 272-281 (Springer-Verlag, Berlin, 1969).
- 23 Nissenbaum, A. & Kaplan, I. R. *Limnol. Oceanogr.* **17**, 570-582.
- 24 Berner, R. A. *Am. J. Sci.* **268**, 1-23 (1970).
- 25 Corliss, J. B. *J. geophys. Res.* **76**, 8128-8138.
- 26 Dymond, J., Corliss, J. B., Heath, G. R., Fisher, C. W., Dasch, E. J. & Veeh, H. H. *Geol. Soc. Am. Bull.* **34**, 3355-3372 (1973).
- 27 Olson, W. S. & Leyden, R. J. *Can. Soc. Petrol. Geol. Mem.* **2**, 720-732.
- 28 Bonatti, E., Ball, M. & Schubert, C. *Naturwissenschaften* **57**, 107-108 (1970).
- 29 Kinsman, D. J. J. in *Petroleum and Global Tectonics* (eds Fischer, A. G. & Judson, S. J.), 84-126 (Princeton University Press, Princeton, N. J., 1973).
- 30 Kinsman, D. J. J. in *4th Symposium on Salt* (ed. Coogan, A. H.), **2**, 255-259 (Northern Ohio Geological Society, Cleveland, Ohio, 1974).
- 31 Young, G. M. *Precambrian Res.* **1**, 13-41 (1974).
- 32 Kulibakina, I. B., Rabotnov, V. T., Goncharov, E. S. & Ahobot, M. R. *Dokl. Akad. Nauk. S.S.S.R.* **201**, 672-674 (Transl. *Dokl. Earth Sci. Ser.* **201**, 86-88, 1971).
- 33 Pisarchik, Y. A. K., Minaeva, M. A. & Rusetskaya, G. A. *Trudy Vses. Issled. Geol. Inst.* **215**, (1975).
- 34 Zharkov, M. A. *Dokl. Akad. Nauk. S.S.S.R.* **184**, 913-914 (1969) (transl. *Dokl. Earth Sci. Ser.* **184**, 72-74, 1969).
- 35 Schindewolf, O. H. & Sellacher, A. *Akad. Wiss. Literat. Mainz Abhandl. Math.-Naturwiss. Kl.*, **1955**, 260-446 (1955).
- 36 Stöcklin, J. *Geol. Soc. Am. Spec. Publ.* **88**, 159-179 (1968).
- 37 Ala, M. A. *Am. Ass. petrol. Geol. Bull.* **58**, 1758-1770 (1974).
- 38 Grayston, L. D., Sherwin, D. F. & Allan, J. F. in *Geological History of Western Canada* (eds McCrossan, R. G. & Glaister, R. P.), 49-59 (Alberta Society of Petroleum Geologists, Calgary, 1964).
- 39 Meyerhoff, A. A. *J. Geol.* **78**, 406-444 (1970).
- 40 Churkin, M., Jr *Am. Ass. petrol. Geol. Mem.* **19**, 485-489 (1973).
- 41 Pilot, J., Rössler, H. V. & Müller, P. *Neue Bergbautech.* **2**, 161-168 (1972).
- 42 Warrington, G. *Qu. J. Geol. Soc. Lond.* **126**, 183-223 (1970).
- 43 Wills, L. J. *Qu. J. Geol. Soc. Lond.* **126**, 225-283 (1970).
- 44 Birkelund, T. & Perch-Nielsen, K. in *Geology of Greenland* (ed. Escher, A. & Watt, W. S.), 305-339 (Greenlands Geologiske Undersogelse, Copenhagen, 1976).
- 45 Richter-Bernberg, G. *UNESCO Earth Sci. Ser.* **7**, 275-287 (1972).
- 46 Degens, E. T. & Stoffers, P. *Nature* **263**, 22-27 (1976).
- 47 Schwarcz, H. P. & Burnie, S. W. *Mineral. Deposita* **8**, 264-277 (1973).
- 48 Knauth, L. P. & Epstein, S. *Geochim. Cosmochim. Acta* **40**, 1095-1108 (1976).
- 49 Buschendorf, F. & Puchelt, H. *Geol. Jahrb.* **82**, 499-582.
- 50 Hoffman, T. *Mineral. Deposita* **4**, 260-274 (1969).
- 51 Reimer, T. *Neues Jahrb. Mineral. Abhandl.* **116**, 167-195 (1972).
- 52 Veizer, J. & Compston, W. *Geochim. Cosmochim. Acta* **38**, 1461-1484.
- 53 Veizer, J. & Compston, W. *Geochim. Cosmochim. Acta* **40**, 905-914 (1976).
- 54 Tremba, E. L., Faure, G., Katsikatos, G. C. & Summerson, C. H. *Chem. Geol.* **16**, 109-120 (1976).

letters to nature

Future variation of Planck's constant

RECENT interest in cosmological models involving time-varying fundamental constants has stimulated experiments to measure such variations which may have occurred in the past. For example, the upper limit to past variations of $\hbar$ relative to other

fundamental constants has been measured as one part in 10^{12}yr^{-1} (refs 1 and 2). The purpose of this letter is to point out that the results of an experiment already performed by Partridge³ for a different reason, can be interpreted as setting a limit to the future variation of $\hbar$ over cosmological times.

In the quantum absorber theory of Hoyle and Narlikar^{4,5},

based on Feynman's path integral approach with a constant $\hbar$, it is the quantum mechanical response of the future part of the universe, rather than local field quantisation, which gives rise to spontaneous emission. This indicates the possibility of measuring future universal behaviour by experiments conducted in the present.

The method of modifying any form of quantum mechanics to allow for a hypothetical variation of $\hbar$ is of course not established experimentally. We can, nevertheless, proceed with reasonable postulates to generalise quantum absorber theory as follows. Consider a charged particle a at time t_0 , which is part of a quantum system such as an atom, in interaction with a similar particle b at some large distance away at a different time t_1 . If the interaction is negligible the quantum behaviour of a is determined by the dimensionless quantity $S_a/\hbar(t_0)$, where S_a is the free action for a and $\hbar(t_0)$ is the value of $\hbar$ at times near t_0 . (We assume the variation of $\hbar$ is small enough for $\hbar$ to be regarded as constant over short laboratory time scales.) A similar quantity $S_b/\hbar(t_1)$ determines the free behaviour of b . When the interaction is not negligible, an interaction term S_I must be added to the action, and the question arises as to what value of $\hbar$ should be used with this term. In a completely time-symmetric universe some symmetric quantity such as $\frac{1}{2}[\hbar^{-1}(t_0) + \hbar^{-1}(t_1)]S_I$ would seem appropriate, but in an asymmetric universe, in which only the future part provides a perfect absorber, it seems preferable to be guided by the self-consistent cycle of argument of Hoyle and Narlikar⁵ in which only retarded fields are used to calculate interactions. Thus if $t_1 < t_0$ the interaction is calculated from $-e\int_{t_0} \hbar^{-1} \mathbf{A}_b^{ret}(\mathbf{a}) \cdot \dot{\mathbf{a}} dt$ where $\mathbf{A}_b^{ret}(\mathbf{a})$ is the retarded field of b acting on a at position $\mathbf{a}$, and, since the integral is taken over an interval near t_0 , it is reasonable to take $\hbar = \hbar(t_0)$. On the other hand when $t_1 > t_0$, as it is when b is one of the particles of the absorber, the appropriate expression is $-e\int_{t_1} \hbar^{-1} \mathbf{A}_a^{ret}(\mathbf{b}) \cdot \dot{\mathbf{b}} dt$ with $\hbar = \hbar(t_1)$.

The values of $\hbar$ can be used to find the influence functional containing the quantum mechanical response of the Universe. The dependence of this functional on $\hbar(t_1)$, the value of $\hbar$ at the time of absorption, arises from the free action terms of the absorber and the interaction terms, and gives the way in which the lifetime of atom a in this modified theory depends upon the epoch at which absorption takes place. An experiment which can compare the transition rate in a source when the source is emitting into a local absorber, for which $\hbar$ takes its present value $\hbar(t_0)$, with the rate when the source is emitting into an absorber at a much greater distance where absorption takes place at time t_1 in the future, would thus give a measure of the future variation of $\hbar$ over the time scale $t_1 - t_0$. Just such an experiment has been done by Partridge³. In this experiment microwaves from a source were alternately directed into a local absorber and into the Universe. For the latter case Partridge estimated that at least 95% of the radiation would reach cosmological distances before absorption. The transition rate from excited states in the source was monitored by measuring the power needed to re-excite the source. Assuming a relation $P_1 = (1 - \delta)P_0$, where P_0 and P_1 represent the power used by the source in the presence and absence of the local absorber respectively, Partridge obtained a value $\delta = (1.1 \pm 1.6) \times 10^{-9}$. Although this experiment was performed for the different purpose of testing the completeness of the universal absorber, and the success in achieving this purpose has been questioned⁶, we can nevertheless use Partridge's data here. Writing $\hbar(t_1) = (1 - \Delta)\hbar(t_0)$ with Δ small, and using $\hbar = \hbar(t_1)$ in the influence functional determining the transition rate, we find that $0.95|\Delta| \approx |\delta|$. Taking the cosmological distances involved to be between 10^9 and 10^{10} light yr at least, gives an upper limit to the variation of $\hbar$ of about one part in 10^{18} yr⁻¹.

This result, which must be one of the longer time constants recorded, would be modified if, for example, the symmetric form of $\hbar$ mentioned earlier were used in the interaction term. The importance of the measurement however, lies not so much in the actual numerical result but in the fact that it is a measurement of the future variation of a physical quantity. It is important to

emphasize that this is not a prediction of future behaviour but, assuming the validity of the theory, is an actual measurement of behaviour which will occur. The apparatus 'sees' into the future.

D. T. PEGG

*J. J. Thomson Physical Laboratory,
University of Reading,
Reading, UK*

Received 7 February; accepted 19 April 1977.

- ¹ Baum, W. A. & Florentin-Nielsen, R. *Astrophys. J.* 209, 319-329 (1976).
- ² Solheim, J. E., Barnes, T. G. & Smith, H. J. *Astrophys. J.* 209, 330-334 (1976).
- ³ Partridge, R. B. *Nature* 244, 263-265 (1973).
- ⁴ Hoyle, F. & Narlikar, J. V. *Nature* 219, 340-341 (1968).
- ⁵ Hoyle, F. & Narlikar, J. V. *Ann. Phys. NY*, 54, 207-239 (1969).
- ⁶ Pegg, D. T. *Nature phys. Sci.* 246, 40-41 (1973).

Astrophysical X-ray spectra: alive and well

CRAIG and Brown¹ have repeated their doubts about the usefulness of high resolution X-ray spectra² and reiterated a series of inaccurate and misleading statements. For example, as early as their second sentence they incorrectly claim that "a high resolution spectrometer is almost invariably included in solar X-ray satellites". High spectral resolution can only be achieved with a collimated spectrometer, the total useful observing time of all such rocket borne devices being currently of the order of an hour. But no solar satellite has ever carried such a spectrometer and only one such device is planned to be in orbit before 1980.

Contrary to the impression given by Craig and Brown, high resolution solar X-ray spectra have found a wide application in the fields of solar physics, atomic physics and astrophysics. High resolution spectrometers are clearly required to resolve line blends, with spectral resolutions of $\lambda/\Delta\lambda \sim 10^3$ being required in some instances (ref. 3 contains several examples). When reliable line intensities have been obtained the atomic physicist has an abundant source of material. In the well known cases concerning Fe XVII (refs 4, 5) and the Helike ion satellite lines⁶⁻⁸, high resolution observations have stimulated formulation of more accurate theories, and progress on many other poorly understood ions is held up by lack of sufficient spectral resolution.

Abundances are fundamental astrophysical parameters that have also been derived from X-ray spectra, not by model-dependent methods as Craig and Brown mistakenly state, but by using line ratios which are insensitive to temperature⁹⁻¹¹. For future experiments further improvements in spectral resolution are planned so that true line profiles can be measured and line widths and shifts investigated.

The main problem worrying Craig and Brown concerns the expression for the intensity of a single spectral line in terms of a Fredholm integral of the type

$$p(a) = \int_b q(b) r(a, b) db$$

Such integrals occur frequently in astrophysics and the problems associated with solving such equations have been extensively discussed. Probably the most commonly encountered form of the above equation occurs in radiative transfer theory when expressing the emergent intensity of a stratified atmosphere in terms of the source function. The elementary approximation of a linear dependence of the source function on the optical depth has little physical significance. Neither does the Eddington-Barbier relationship which is subsequently derived, but the usefulness and accuracy of this end result cannot be disputed.

Given the inherent mathematical restrictions relating to the form of the above equation, progress on the astrophysical problem can only be made by adopting one of the following

procedures: (1) The use of a sophisticated matrix inversion technique, which helps to overcome the problems of 'bad' data and a limited range of observations, for example, Phillips-Twomey, Backus-Gilbert. (2) The derivation of a 'best fit' model with no *a priori* assumptions about its structure^{3,12,13}. (3) The derivation of a 'best fit' model with a predetermined analytical structure¹⁴⁻¹⁶.

At present solar X-ray observations are not of sufficient quality to justify the use of procedure (1). There is nothing inherently wrong with a 'best fit' model provided its limitations are recognised and it is used sensibly in its relevant domain. In fact most astrophysical models have been constructed using either (2) or (3) above: only the simplified situations seem to have exact solutions. Thus 'best fit' active region models are presently no less accurate than many other astrophysical models and they do have every prospect of improving when observations of more lines covering a wider temperature range become available.

Craig and Brown have used an elementary simulation to predict the intensities of several lines for an assumed structure. These line intensities are given a random perturbation and a model is computed. However, they overlook the fact that emission measures are everywhere defined as positive quantities. The problem of negative emission measures arises from using pairs of lines with broadly similar temperature responses such that small perturbations in the data lead to inverted flux ratios. Whilst it is true that none of the computed models seem to resemble the input structure, a simple averaging of these models, that is, a 'best fit' (or at least a 'better fit') gives a model closely resembling the input structure, indicating that a convergent (self-consistent) solution to the problem is possible.

In conclusion, it is safe to say that the interpretation of X-ray spectra is alive and well, and will continue to thrive with improved instrumentation.

'Best fit' models for solar active regions are just as accurate as any other astrophysical models and far from being 'spurious' are found to be in agreement with others derived from ultraviolet and optical data. X-ray astronomy in general, after several initial breakthroughs with 'quite simple detectors' will continue to make progress with telescopes having improved spatial resolution and spectrometers with improved spectral resolution.

JOHN H. PARKINSON

Mullard Space Science Laboratory,
University College London,
Holmbury St Mary,
Dorking, Surrey, UK

Received 17 December 1976; accepted 15 April 1977.

- ¹ Craig, I. J. D. & Brown, J. C. *Nature* **264**, 340-342 (1976).
- ² Craig, I. J. D. & Brown, J. C. *Astron. Astrophys.* **49**, 239-250 (1976).
- ³ Parkinson, J. H. *Solar Phys.* **42**, 183-207 (1975).
- ⁴ Loulergue, M. & Nussbaumer, H. *Astron. Astrophys.* **24**, 209-213 (1973); **45**, 125-134 (1975).
- ⁵ Parkinson, J. H. *Astron. Astrophys.* **24**, 215-218 (1973).
- ⁶ Parkinson, J. H. *Nature phys. Sci.* **233**, 44-45 (1971); **236**, 68-70 (1972).
- ⁷ Gabriel, A. H. *Mon. Not. R. astr. Soc.* **160**, 99-119 (1972).
- ⁸ Bhalla, C. P., Gabriel, A. H. & Presnyakov, L. P. *Mon. Not. R. astr. Soc.* **172**, 359-375 (1975).
- ⁹ Acton, L. W., Catura, R. C. & Joki, E. G. *Astrophys. J.* **195**, L93-L95 (1975).
- ¹⁰ Ruge, H. R. & Walker, A. B. C. *Astrophys. J.* **203**, L139-L143 (1976).
- ¹¹ Parkinson, J. H. *Astron. Astrophys.* (in the press).
- ¹² Batstone, R. M., Evans, K., Parkinson, J. H. & Pounds, K. A. *Solar Phys.* **13**, 389-400 (1970).
- ¹³ Jordan, C. *IAU Symp.* **68**, 109-131 (1975).
- ¹⁴ Chambe, G., *Astron. Astrophys.* **12**, 210 (1971).
- ¹⁵ Acton, L. W., et al. *Solar Phys.* **26**, 183-201 (1972).
- ¹⁶ Walker, A. B. C., Ruge, H. R. & Weiss, K. *Astrophys. J.* **188**, 423-440 (1974); **192**, 169-180 (1974).

those involved in space experiments, but their statements represent only one extreme viewpoint on this complex problem.

The main thrust of their argument is to point out the ill-conditioning of the equations that require to be inverted in order to carry out an analysis based on emission measures, and to illustrate this by reference to a 1970 publication by Batstone *et al.*², an early attempt to do this in the X-ray region. It should be pointed out that emission measure analysis is only a small (though important) part of the full capability of spectroscopic diagnostics; that nowadays it is possible to combine it with spatial and time resolution of the source, and that the 1970 work is in any case not as unsound as they suggest.

To start with the last point, it is basic to all interpretational physics that the aim is to find the simplest model consistent with the observations. To insist, as do Craig and Brown, that all possible models, however complex, are equally valid and worthy of consideration would have seriously impeded progress in any branch of physics to which it had been applied. If we consider the histogram of emission measure X against temperature T from their example in terms of its Fourier components in harmonics of the temperature parameter, then it is clear that the simplest model is one with only low frequency components. The solution derived by Batstone *et al.*² is a monotonic decrease from $T = 1$ to $T = 10$. Craig and Brown's alternative valid solutions all have for their largest amplitude Fourier component the highest harmonic permitted by the temperature interval— X oscillates violently up and down between each temperature point. Most physicists will recognise the unique nature of the simplest solution proposed by Batstone *et al.* in contrast to the contrived complexity of the alternatives. Furthermore, if we apply a simple smoothing (averaging pairs of adjacent points) all four models become very similar. Thus if we remove the highest Fourier component (normally spurious when fitting data based on discrete intervals) the physical reality of what is left is manifest by the fact that Craig and Brown's models, derived specifically to demonstrate the variability, tend to approach the same solution. The exception is the lowest temperature point, which remains variable to some extent due to the simple physical fact that the experiment is not very sensitive at these low temperatures. Craig and Brown, although right in demanding some form of error estimates in published data, are wrong in assuming that the full curve with all its temperature points is required in order to derive any significant conclusions from the data, and are also wrong in claiming that a single source temperature and variance could equally well describe the model. The significant data from this particular spectrum is probably describable by at most three points on an X against T curve, with the assumption of monotonic variation between them, and an asymptote at high T of zero. This is perhaps the limit of the information content, but such information can be very valuable. With modern space instrumentation techniques, data of this type can be obtained for a solar structure as a function both of position in two coordinates, and of time. Such a matrix of observations would provide a formidable challenge in the fitting together of theoretical and observational models. In practice, such a measurement nowadays would be likely to cover a much wider range of temperatures, and thereby further enhance its usefulness.

Emission measure analysis has long been used in the ultraviolet region for solar (and stellar) chromosphere structure determinations³. I have drawn attention⁴ to the limitations of this technique. In the ultraviolet case, however, the technique is much more precise than for X rays from active regions, and the limitations referred to above relate only to fine detail in the X against T curve. Craig and Brown claim that the method is always ill-conditioned due to the broad form of the kernel function F ; or to use the more usual Pottasch⁵ notation, the temperature dependent line factor $g(T)$. They claim that this is because F is always dominated by the Maxwellian electron distribution factor, $\exp(-E/kT)$. In this they are wrong. F contains the ion stage population N_i/N_e which is built up from

Why measure astrophysical X-ray spectra?

CRAIG and BROWN¹ advise caution against some of the limitations of X-ray astronomy, and emphasise the need to take theoretical considerations into account before planning expensive experiments. Their remarks must be taken seriously by

the product of many functions of the type $\exp(-E_i/kT)$ and consequently falls off much faster than a single function of this type, and in a way which varies with the wavelength region and type of ion chosen.

The above situation can be recognised more physically if we make an approximation to equation (1) of Craig and Brown

$$I(\epsilon) = \int_T \xi(T) F(\epsilon, T) dT \quad (1)$$

If the functions are transformed to a logarithmic temperature scale, so that $\theta = \log T$, then the kernel $F(\epsilon, \theta)$ can usually be approximated by a constant-shaped function, centred on a temperature θ_ϵ which is a function of the particular line ϵ . This is particularly true for the case dealt with by Batstone *et al.*², in which the lines are mostly isoelectronic, that is, they have similar atomic parameters, excitation energies and so on in relation to the charge on the ion. The equation can then be rewritten

$$I(\epsilon) = \int_{\theta} \xi(\theta) F(\theta - \theta_\epsilon) d\theta \quad (2)$$

Equation (2) can be recognised as a simple convolution integral in which the observable $I(\epsilon)$ is the result of folding the unknown $\xi(\theta)$ with a constant 'instrumental' profile $F(\theta)$. Its inversion properties are well-known; $\xi(\theta)$ can be derived from $I(\epsilon)$ reliably in regard to structure which is on a θ scale large compared with the width of $F(\theta)$, but becomes very ill-conditioned when trying to extract detail on a smaller scale than the width of $F(\theta)$. This simple approximation is useful to the physicist in understanding when the inversion of equation (1) is valid, and the inherent limitations in the solution obtained.

I now add a few remarks in defence of high wavelength resolution spectroscopy. The separation with confidence of spectral line blends into discrete lines opens up wide possibilities for the use of line ratios which, through the physics of the excitation mechanism, can be interpreted in terms of local electron temperature and density. This method can be much more precise than emission measure analysis, but is at present limited in its scope by limitations in the available atomic collision rates. The excitation process has a shorter relaxation time-constant in the plasma than emission measures which depend on ionisation balance. Consequently, the combined use of line ratios and emission measures can give vital information on the transient state of the plasma, that is the rate at which it is ionising or recombining. For solar X-ray measurements nowadays, lines and continuum can usually be separated. But, because of the lower fluxes, cosmic X-ray measurements so far have generally been broad-band, using the proportional counter as a spectrometer. Consequently, the expected strong line groups appear only as a bump in the spectrum, and it may be suspected that many of the 'continuum' measurements are contaminated by un-resolved lines. To suggest as do Craig and Brown that such crude detectors are more than adequate is therefore incorrect. When a future generation of cosmic X-ray spectrometers is able to resolve and separate lines, a whole new dimension of diagnostics will become possible and we can look forward to dramatic advances in our understanding of the recently discovered objects.

In conclusion, the field of spectroscopic diagnostics of space plasmas has come a very long way since 1970, when the emission measure analysis of a source, smeared over space and time, was a new development. We can now make detailed and precise measurements of a range of physical properties. Unfortunately, this calls for complex and sophisticated observational techniques, involving high spectral, space, and time resolutions and increased sensitivities. The criticisms levelled at emission measure analysis by Craig and Brown are not all sound, and ignore the proper role of such techniques within a broader

base which includes a range of more sophisticated techniques. Space experiments are not nowadays designed and built without input from theory. Most of the consortia currently in this field include members well versed in the present status of theoretical models, and with a broad understanding of the range of sophisticated diagnostic techniques currently available.

A. H. GABRIEL

*Appleton Laboratory Astrophysics Research Division,
Culham Laboratory,
Abingdon, Oxon, UK*

Received 6 December 1976; accepted 15 April 1977.

- ¹ Craig, I. J. D. & Brown, J. C. *Nature* **264**, 340–342 (1976).
- ² Batstone, R. M., Evans, K., Parkinson, J. H. & Pounds, K. A. *Solar Phys.* **13**, 389–400 (1970).
- ³ Jordan, C. & Wilson, R. in *Physics of the Solar Corona* (ed. Macris, X.) (Reidel, Boston, 1971).
- ⁴ Gabriel, A. H. in *Proc. IAU Coll. No. 36, The Energy Balance and Hydrodynamics of the Solar Chromosphere and Corona* (ed. Bonnet) (in the press).
- ⁵ Pottasch, S. R. *Astrophys. J.* **137**, 945 (1963).

BROWN AND CRAIG REPLY—We do not refute the value of very high resolution spectrometry to atomic physics, nor in astrophysical abundance and line profile studies. However, grant proposals for satellite spectrometers of $\lambda/\Delta\lambda \approx 10^3$ usually identify $\xi(T)$ measurement as a primary aim (recognising that atomic line identification is harder to justify in an astronomy budget). Concerning the inference of $\xi(T)$ we reply:

Simplicity of models?—Gabriel's point of smoothness in $\xi(T)$ is well taken but a half sine wave in $(1-10) \times 10^6$ K fits the data and is simpler (one Fourier term less) than his "unique simplest solution" without remotely resembling it. It is also positive everywhere, which may please our critics, but so are about 500 other diverse meaningless solutions we generated¹ which fit the data. Strangely both authors claim convergence of our $\xi(T)$ from three random terms² when we proved¹ non-convergence after 10^4 terms! We agree with Parkinson that AR analyses may be "no less accurate than many other astrophysical models" and indeed recommend close scrutiny of the non-uniqueness of all such. Astrophysical plasmas are regrettably unaware that they should be smooth and simple^{4,5}.

Validity of analysis?—Gabriel's reference to convolutions and Parkinson's to radiative transfer integrals in fact amply vindicate our analysis. The extreme width of $F(\theta)$ in the former proves⁶ that $\xi(T)$ can only ever be crudely resolved so that high spectral resolution is futile for this purpose. Would Gabriel kindly reference one line whose temperature response width $\Delta T \ll \epsilon_{\text{line}}/k$? Parkinson correctly states that radiative transfer theorists regularly use linear source function approximations but they always emphasise that this crude result cannot be much improved by increasing data resolution (ref. 7 and J. T. Jefferies and Skumamich, personal communication) (for example more limb darkening coefficients)—precisely our point. We are well aware⁶ of the 'more refined' data reduction techniques possible but our critics seem unaware that these all heavily use *a priori* assumptions about the solution to augment the low information content of the experiment^{6,8}.

Originality and accuracy?—As far as we know we were first to: unambiguously define $\xi(T)$ physically; recognise the X-ray spectrum problem as a Fredholm equation and prove it ill-posed; use condition number analysis as a measure of maximum useful resolution in astrophysics. Fortunately others (for example ref. 7) do not share Parkinson's view that we have "added nothing new" and our analysis has indeed been used in Skylab data reduction⁸ and in Spacelab proposals. As to our alleged inaccuracy, Parkinson's opening example seems to be of semantic origin for in this context "high resolution" evidently means 10^4 – 10^5 whereas by his second paragraph "high resolution" has been reduced to $\sim 10^3$. Though for $\xi(T)$ analysis

even spectral resolutions greater than 10^1 – 10^2 are “high”, spectrometers of $\lambda/\Delta\lambda < 10^3$ were in fact in orbit⁹ as early as 1967.

I. J. D. CRAIG
J. E. BROWN

Department of Astronomy,
University of Glasgow,
Glasgow, UK

- ¹ Craig, I. J. D. & Brown, J. C. *Astron. Astrophys.* **49**, 239 (1976).
- ² Craig, I. J. D. & Brown, J. C. *Nature* **264**, 340–342 (1976).
- ³ Craig, I. J. D. *Astron. Astrophys.* (in the press).
- ⁴ Craig, I. J. D. & McClymont, A. N. *Solar Phys.* **50**, 133 (1976).
- ⁵ Herring, J. F. *Solar Phys.* **39**, 175 (1974).
- ⁶ Bohm, K. H. *Astrophys. J.* **134**, 264 (1961).
- ⁷ Underwood, J. H. & McKenzie, D. L. *Solar Phys.* (in the press).
- ⁸ Turchin, V. F., Kezlov, V. P. & Malkevich, M. S. *Soviet Phys. Usp.* **13**, 681 (1971).
- ⁹ Meekings, J. F., Doschek, G. A., Friedman, H., Chubb, T. A. & Kreplin, R. W. *Solar Phys.* **13**, 198 (1970).

Night-time reception of solar radio events

RIIHMAA has reported night-time reception of a solar radio event¹. The observation, in the frequency range 20.85–23.20 MHz, was made at Kiiminki (65°05'N, 25°54'E) on 30 March 1976 with the Sun (and Jupiter) well below the horizon. Night-time reception of solar bursts had also been reported by Smith *et al.*². Such night-time solar radio burst reception might be brought about by anomalous propagation associated with plasma waves in the lower ionospheric E layer. I briefly discuss this phenomenon here and point out the possible importance of large ionospheric electric fields.

Riihmaa¹ argues that “in the present case the bursts must have been guided from the sunlit hemisphere over the polar regions. It may be significant that on the night of 30 March an auroral substorm and a strong blanketing sporadic-E (E_s) were observed. A suitable duct may have existed between the ground and the E_s layer.” The total attenuation was estimated to be of the order of ≈ 10 dB (ref. 1 and personal communication).

The question arises as to what mechanism(s) may bring about the anomalous propagation of the bursts into the night-time hemisphere. Since ray bending and geometrical optics considerations do not seem to suffice, one may resort to a slightly more sophisticated approach.

An E layer in which electric fields above a nominal threshold of ~ 25 mV m⁻¹ excite Farley–Buneman waves^{3,4} of sufficiently large amplitude, may provide enough reflectivity to the ~ 20 MHz radiation and ‘guide’ it over the polar region into the night-time hemisphere. I have considered the reflectivity of a Farley–Buneman unstable E layer in a brief paper on riometers⁵. For details see ref. 5 and refs therein.

In the present connection we need only note the following. Reflection of 20-MHz radiation (wavelength $\lambda = 15$ m) by a system of plane parallel plasma waves occurs by Bragg scattering when the relation $\lambda = 2\lambda_n \cos\theta$ is satisfied, λ_n being the relevant wavelength of the plasma waves and θ the angle of incidence. With $\alpha = \pi/2 - \theta \ll 1$ the above relation reads $\lambda \approx 2\lambda_n \sin\alpha \approx 2\lambda_n\alpha$. A typical angle α under which 20-MHz ‘rays’ from the Sun ‘graze’ from below the bottom part of the polar cap E layer and are reflected into the night-time hemisphere is $\alpha \sim 0.2$ rad $\sim 10^\circ$. Then the appropriate plasma wavelength for reflection is $\lambda_n \approx 40$ m. Plasma wavelengths of ~ 40 m (in the present case the geometry is such that λ_n is to be understood as the plasma wavelength along the geomagnetic field lines) are available among the Farley–Buneman waves generated by large electric fields in the E layer (ref. 5 and refs therein).

To compute the efficiency of reflection is a difficult task, but some estimates can be made. If we use equation (1) of ref. 5 for the (power) reflectivity R , a plasma frequency $f_p = \omega_p/2\pi \approx 4$ MHz, a percentage plasma density modulation $|\Delta n/n| \sim 0.1$, and a number of scatterers along the ray path $N \sim 10^4$ m per

40 m $\sim 2.5 \times 10^2$, we find $R \approx 6\%$, that is a reflectivity high enough for this reflection process to be of possible significance in ‘guiding’ the 20-MHz radiation into the high latitude night-side. Note that only one or at most two reflections from the ionosphere are required.

It seems of some relevance that, at the time of Riihmaa’s observation, the K_p index was 4⁻, indicative of polar cap electric fields above the Farley–Buneman threshold. The possibility arises of testing the proposed mechanism by determining whether night-time reception of solar radio bursts occurs preferentially when large ionospheric electric fields exist over the polar cap. Were a correlation to be found, it would be in line with the observed correlation between high intensity polar cap electric fields and the occurrence of the Slant E Condition (SEC) in high latitude ionograms^{6,7}.

I thank Dr J. J. Riihmaa for helpful comments.

N. D’ANGELO

Department of Physics and Astronomy,
University of Iowa,
Iowa City, Iowa 52242

Received 7 March; accepted 6 April 1977.

- ¹ Riihmaa, J. J. *Nature* **263**, 397 (1976).
- ² Smith, A. G., Carr, T. D. & Perkins, W. H. *Nature* **183**, 597 (1959).
- ³ Farley, D. T. *J. geophys. Res.* **68**, 6083 (1963).
- ⁴ Buneman, O. *Phys. Rev. Lett.* **10**, 285 (1963).
- ⁵ D’Angelo, N. J. *J. geophys. Res.* **81**, 5581 (1976).
- ⁶ D’Angelo, N. & Olesen, J. K. *J. geophys. Res.* **80**, 2866 (1975).
- ⁷ Iversen, I. B., D’Angelo, N. & Olesen, J. K. *J. geophys. Res.* **80**, 3713 (1975).

Why is the Maier–Saupe theory of nematic liquid crystals so successful?

THE Maier–Saupe theory of nematic liquid crystals is founded on a molecular field treatment of long-range contributions to the intermolecular potential and ignores the important short-range forces. Nonetheless this theory is particularly successful in predicting the orientational properties of real nematics. Here we propose a model which accounts both for the success of the Maier–Saupe theory and the failure of theories based exclusively on short-range forces.

The liquid crystal phase of a nematogen is characterised by the long-range orientational order which is destroyed at the transition to the isotropic fluid. The Maier–Saupe theory has been particularly successful in accounting, not only for the order–disorder transition, but also for the orientational properties of the nematic mesophase¹. The theory is founded on the molecular field approximation applied to a weak anisotropic pair potential; this then gives the effective orientational potential for a single particle as

$$U(\cos\theta) = \bar{u}_2 \bar{P}_2 P_2(\cos\theta)$$

where θ is the angle between the molecular symmetry axis and the director. The order parameter, $\bar{P}_2$, is the ensemble average of the second Legendre polynomial and $\bar{u}_2$ is a combination of the averaged anisotropic interaction parameters. Because the theory contains just one unknown, $\bar{u}_2$, the change at the transition in the order parameter ($\bar{P}_2^{(K)}$) and the entropy ($\Delta S^{(K)}$) are predicted to be universal properties of the nematogen. This prediction is in reasonable agreement with experiment; in addition the calculated values for $\bar{P}_2^{(K)}$ of 0.43 and $\Delta S^{(K)}/R$ of 0.417 are found to be in quite good accord with the observed values^{2,3}. The agreement with experiment can be made perfect by extending the Maier–Saupe theory in a variety of ways. These include the addition of terms to the intermolecular potential to allow for higher rank interactions⁴ and deviations from molecular cylindrical symmetry⁵ as well as by taking the molecular field approximation to higher order⁶.

We shall not consider these extensions, however, because our aim is to understand the reasons for the success of the Maier-Saupe theory rather than to improve it further. In particular we shall consider whether its achievements are accidental or whether they provide an insight into the role of short- and long-range forces in establishing nematic order. Despite its qualitative and even semi-quantitative success the Maier-Saupe theory has been widely criticised (refs 7, 8 and M. A. Cotter, in preparation). One of the major criticisms stems from the foundation of the theory on an anisotropic pair potential which is apparently determined solely by London dispersion forces⁷. As a consequence the nematic-isotropic transition temperature for an ensemble of rod-like particles is predicted to be^{1,4}

$$T_K = 4.541 \times 3h\nu\Delta\alpha^2/20k r^6$$

It is possible therefore to test the theory by attempting to calculate the transition temperature from a knowledge of the anisotropy, $\Delta\alpha$, in the molecular polarisability tensor. Thus order of magnitude estimates of $\Delta\alpha$ and the dispersive energy are found to yield transition temperatures which are considerably lower than those observed^{7,8}. Of course these estimates are themselves open to criticism because the internal electric field in an anisotropic medium is not well understood⁹. None-the-less it is doubtful if dispersion forces, as envisaged by Maier and Saupe, could be entirely responsible for the existence of liquid crystals.

A second attack on the theory is concerned with its complete neglect of anisotropic short-range repulsive forces which should be of special importance for the elongated molecules forming nematogens. This neglect would seem to be quite unjustified as it is now widely accepted that scalar repulsive forces have a dominant role in determining the organisation in simple fluids¹⁰. Such considerations have prompted the development of theories based on purely short-range anisotropic forces using a variety of approximations, which include the Onsager approach¹¹, lattice summations¹² and scaled particle theory¹³. In every case the order parameter and entropy change at the predicted phase transition were found to be much larger than their experimental counterparts. For example, the Onsager theory¹¹ gives the order parameter $\bar{P}_2^{(K)}$ at the transition as 0.85 which is over twice the value normally observed for nematogens while the entropy change $\Delta S^{(K)}/R$ is predicted to be 8 which is in marked contrast to the observed values of about 0.3 (refs 2, 3). Of course the high length-to-breadth ratio of the constituent particles in the systems discussed by Onsager, are not realistic for a nematic, however the scaled particle theories do not suffer from this fault, and in addition, have been reasonably successful when applied to simple fluids¹⁰. None-the-less the predicted values of $\bar{P}_2^{(K)}$ are still found to be in excess of those determined by experiment, similarly the discontinuity in the density at the order-disorder transition is also predicted to be too large¹³. Such failings do not seem to be a consequence of the necessary approximations inherent in the theories for a computer-simulation experiment based on a continuous repulsive potential also yields an unrealistically high value for the order parameter¹⁴.

The theories based on interactions between hard particles suffer from another weakness, namely the absence of any direct dependence of the orientational properties on temperature. Thus, even though it may be reasonable to suppose that the order-disorder transition depends solely on density for a suspension of macromolecules, it is difficult to believe that density alone determines the transition in thermotropic liquid crystals. Indeed this failure is highlighted by the inability of these theories to account for the observed temperature dependence of the order parameter, $\bar{P}_2$, at constant volume¹⁵. In marked contrast the Maier-Saupe theory is able to provide a temperature dependence in very good agreement with experiment¹⁶.

We are faced, therefore, with an apparent dilemma. On the one hand theories based on short-range repulsive forces, which are expected to make the dominant contribution to the pair

potential, yield results in poor accord with experiment. While on the other hand, the Maier-Saupe theory, founded on long-range dispersion forces, provides an excellent description of the orientational properties of real nematogens. A formal solution to this dilemma is provided by the molecular field theories based on a completely general form of the total intermolecular potential¹. This is separated into a scalar component and an anisotropic part¹⁷ which is then written in terms of the Pople expansion. The orientational part of the single particle potential is found to be a series whose first term has the same form as the Maier-Saupe result, although the coefficient $\bar{u}_2$ now contains, in principle, contributions from all anisotropic forces. The rapid convergence of the expansion for the single-particle potential is guaranteed, at least for nematogens, by the marked decrease of the general order parameter, $\bar{P}_L$, with its rank L . Consequently, it is not necessary for the Pople expansion itself to converge rapidly; indeed, for small intermolecular separations the convergence is slow. The Maier-Saupe theory is, therefore, equivalent in form to one which includes both long- and short-range contributions to the anisotropic intermolecular potential.

There are, however, several unsatisfactory features of this analysis. In the first instance because all intermolecular forces contribute to $\bar{u}_2$ this is virtually impossible to determine and so the theory cannot be tested by calculating the nematic-isotropic transition temperature. By the same token, we are unable to discern which anisotropic forces are the most important for the formation of nematic liquid crystals. Finally, the general theory shows that the distance dependence of the anisotropic potential must be averaged over the pair distribution function appropriate for particles interacting with a purely scalar potential¹⁷. Such an averaging procedure seems physically unrealistic and so quite different schemes have been devised which allow for the anisotropy of the short-range forces.

Here we propose a solution to these difficulties. We suggest that both short- and long-range forces are important in determining the molecular organisation in a nematic phase but that they operate at quite different levels. In our model we suppose that the short-range forces are responsible for the formation of highly ordered groups or clusters of molecules. These forces may be repulsive as suggested by the behaviour of simple fluids, although with molecules as complex as those in nematogens specific anisotropic electronic interactions could also be involved. Physically we might imagine the cluster to be less anisometric than its constituent particles; as a consequence the anisotropic short-range forces between clusters would become of minor importance. Secondly, the anisotropy in the polarisability of the cluster will be approximately proportional to the number of particles in the cluster and so the dispersive interaction energy between clusters would become dominant. The net effect of the existence of clusters would be to reduce the anisotropy associated with the short-range forces while increasing the influence of long-range forces. The possibility of cluster formation was mentioned in the original Maier-Saupe theory¹ although its purpose seems to have been to force agreement between the predicted and observed entropy of transition. The other important ingredient of our model is the assumption that the clusters are not destroyed at the nematic-isotropic phase transition. The anisotropic forces between clusters are therefore responsible for the orientational properties of the nematic mesophase. The parameter $\bar{u}_2$ in the effective potential is not then determined by the interaction of two molecules, but by two clusters. Consequently, the coefficient $\bar{u}_2$ will be essentially proportional to the square of the number of particles in a cluster and so, even if dispersion forces did dominate the long-range interactions, it would still be possible to produce the necessary increase in the predicted transition temperature by the inclusion of a relatively small number of particles in a cluster. We can also understand the failure of theories based on repulsive forces. The fundamental unit in these calculations is a single particle and at the transition the orientational order is completely destroyed which produces a large increase in entropy. Whereas for the cluster model the transition unlocks the

ordering of the clusters but not that of the molecules within the cluster; consequently, the entropy of transition is small.

The notion of cluster formation in nematogens is apparently of value. It is important, however, to appreciate exactly what is meant by a cluster; indeed, such a concept has already been questioned because it was thought that the cluster should have some physical existence, as an independent object⁸. This existence is not necessary. In the language of statistical mechanics the cluster would be described in terms of the spatial and orientational pair distribution function. Then our model is equivalent to the assumption that the distribution function exhibits short-range order, over several molecular distances, and this order is not destroyed at the nematic-isotropic transition. Consequently, the variation in the orientational properties on passing from one phase to the other will be determined by the long-range intermolecular forces corresponding to distances over which the distribution function does change.

Finally, we must see if there is any other evidence, apart from the success of the Maier-Saupe theory and the failure of hard particle treatments, with which to support our model. Clearly, X-ray and neutron scattering studies are of particular significance because the scattering pattern is directly related to the distribution functions. Such experiments have been performed and they do support the cluster model. For example, the average intermolecular spacing is found from X-ray studies to be about 5.0 Å; this spacing exhibits a weak temperature dependence and is continuous at the nematic-isotropic transition¹⁸. In addition, the high local molecular order is also found to extend for about five molecular diameters but to be insensitive to temperature even at the transition. Preliminary investigations of 4,4'-dimethoxyazobenzene based on coherent neutron scattering also indicate the existence of high local ordering present in both nematic and isotropic phases but with the molecular planes stacked parallel¹⁹. There is additional, although less direct evidence for the existence and preservation of clusters above and below the phase transition. Thus the far-infrared or Poley absorption of 4-methoxybenzylidene-4'-n-butylaniline exhibits only a minor change in frequency as the temperature is varied through the order-disorder transition²⁰. Since the absorption frequency is determined by the short-range contributions to the intermolecular potential, these results imply that the short-range part of the distribution function does not change when the ordered phase is destroyed.

In conclusion, we assert that both short and long-range forces are important in determining the molecular organisation in a nematic liquid crystal. But, because the short-range part of the pair distribution function does not change appreciably at the nematic-isotropic transition, it is the long-range contribution to the pair potential which determines this transition. Consequently, the Maier-Saupe theory, founded on such forces, provides a good description of nematic liquid crystals, whereas theories based on short-range forces are invariably less successful.

G. R. LUCKHURST
C. ZANNONI

Department of Chemistry,
The University,
Southampton, UK

Received 16 March; accepted 14 April 1977.

- ¹ Maier, W. & Saupe, A. Z. *Naturforsch.* 13a, 564-570 (1959); 14a, 882-900 (1959); 15a, 287-292 (1960).
- ² Saupe, A. (ed.) *Angew. Chem. int.* 7, 97-118 (1968).
- ³ Faber, T. E. & Luckhurst, G. R. A. Rep., A72, 31-65 (1975).
- ⁴ Humphries, R. L., James, P. G. & Luckhurst, G. R., *J. Chem. Soc. Faraday Trans. II* 68, 1031-1044 (1972).
- ⁵ Luckhurst, G. R., Zannoni, C., Nordio, P. L. & Segre, U. *Molec. Phys.* 30, 1345-1358 (1975).
- ⁶ Sheng, P. & Wojtowicz, P. J. *Phys. Rev. A* 14, 1883-1894 (1976).
- ⁷ Kaplan, J. L. & Drauglis, E. *Chem. Phys. Lett.* 9, 645 (1971).
- ⁸ Wulf, A. J. *chem. Phys.* 64, 104-109 (1976).
- ⁹ Dunmur, D. A. *Molec. Phys.* 23, 109-115 (1972).
- ¹⁰ Andersen, H. C., Chandler, D. & Weeks, J. D. *Adv. chem. Phys.* 34, 105-156 (1976).
- ¹¹ Onsager, L. *Ann. N.Y. Acad. Sci.* 51, 627-659 (1949).
- ¹² Alben, R. *Molec. Cryst. Liq. Cryst.* 13, 193-203 (1971).
- ¹³ Cotter, M. A. *Phys. Rev. A* 10, 625-636 (1974).
- ¹⁴ Kushick, J. & Berne, B. J. *J. chem Phys.*, 64, 1362-1367 (1976).
- ¹⁵ McColl, J. R. & Shih, C. S. *Phys. Rev. Lett.* 29, 85-87 (1972).
- ¹⁶ Humphries, R. L. & Luckhurst, G. R. *Chem. Phys. Lett.* 17, 514-515 (1972).
- ¹⁷ Zannoni, C. thesis, Univ. Southampton (1975).
- ¹⁸ Leadbetter, A. J., Richardson, R. M. & Colling, N. J. *Phys. (Paris)* 36, C1 37-43 (1975).
- ¹⁹ Niimura, N. *Molec. Cryst. Liq. Cryst.* 31, 123-130 (1975).
- ²⁰ Evans, M., Davies, M. & Larkin, I. J. *Chem. Soc., Faraday Trans. II* 69, 1011-1022 (1973).

New shock effects in the phosphide and carbide phases of Cañon Diablo iron meteorites

WE report a hitherto unrecorded variety of shock-induced damage in the cohenite, (FeNi)₃C, of a 'rim type' specimen of Cañon Diablo. The essential feature of the new effect is the wedge-shaped area of cloudy appearance that penetrates into the cohenite from the original cohenite-kamacite interface (see Fig. 1). The figure also shows the reheated, α_2 , condition of the kamacite and the dark-etching zone of carbon diffusion at the cohenite-kamacite interface; effects that have often been reported in 'rim type' samples of Cañon Diablo¹⁻³ and have been produced artificially by shock¹ or isothermal reheating⁴.

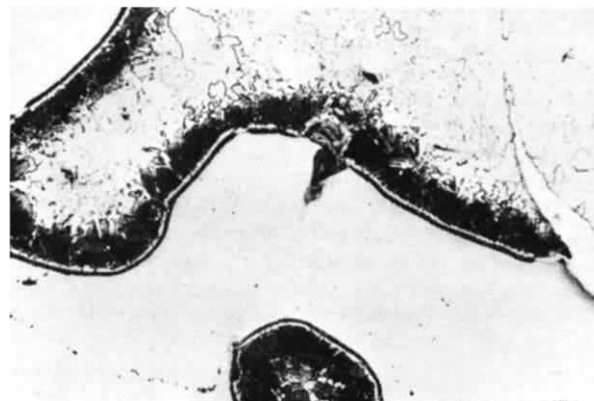


Fig. 1 Etched 1% Nital. Dark etching wedge of cloudy material penetrates structureless white cohenite. α_2 matrix, white, granular. Black carbon diffusion border at cohenite- α_2 interface. Note small unaltered phosphide on outer elbow of cohenite near centre of field. Field of view 0.63×0.42 mm.

Figure 2 represents the same feature at higher magnification and shows a characteristic pattern of swirls within the cloudy area.

Figure 3 is a scanning electron micrograph which demonstrates the duplex condition of the cloudy area. Microprobe point analyses of the light background in Fig. 3 were erratic but generally similar to unaltered cohenite, whereas point analyses of the dark inclusions consistently gave slightly higher nickel values. No phosphorus was detected within the cloudy area.

These observations were made on polished and etched sections of a 147-g specimen (number H 37.546 purchased from the American Meteorite Laboratory) and within this specimen numerous examples of the effect were encountered. Some of the cloudy areas in the cohenite were associated with incompletely reacted remnants of phosphide, which commonly occurs as small particles at the cohenite-kamacite interface. Larger areas of cloudy material extend deep into the cohenite crystals and sometimes completely transect them. In specimen H 37.546 these larger areas of cloudy material are occasionally accompanied by microscopically visible decomposition of the cohenite to graphite.

Under polarised light the cohenite appears polycrystalline but shows accentuated recrystallisation along the trace of

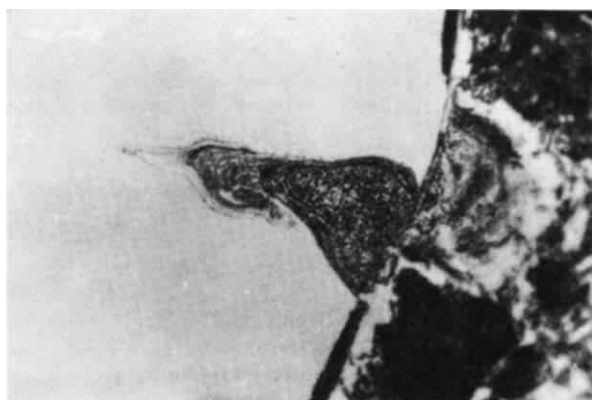


Fig. 2 Detail from Fig. 1 showing swirls within the cloudy wedge. Field of view 0.16×0.10 mm.

shear zones. Visible shear displacements were observed in the cohenite and in taenite. In the bulk kamacite any possible shear traces were obscured by the reheated, α_2 , condition but a small globule of kamacite that was enclosed by cohenite showed a sharply defined shear displacement that lay along a line of enhanced recrystallisation in the surrounding cohenite. Specimen H 37.546 also contained occasional filaments of phosphide located between adjacent kamacite bands. In most instances the only indication of shock reheating shown by this phosphide was the development of minor thorns⁴ at the phosphide–kamacite interface. However, a portion of this phosphide was found to show a fine duplex appearance similar to the cloudy areas observed in the cohenite. Microprobe analysis of this material showed no detectable change of nickel or phosphorus content when there was no detectable extension of the material beyond the original phosphide–kamacite interface. This effect may be called '*in-situ*' melting of the phosphide; it appeared to be distributed along shear zones in the specimen and it has probably resulted from the effect of local shear superimposed on a general level of shock that was just insufficient to induce general melting of the phosphide.

The combination of shear, shock and reheating effects observed in this specimen are consistent with the following interpretation of the wedges of cloudy material observed in cohenite, Figs 1–3. The average or bulk level of shock in this specimen was not sufficient to induce melting of phosphide at the kamacite grain boundaries nor of the smaller phosphide particles at cohenite–kamacite interfaces. However, local shear, superimposed on the overall shock, provoked local '*in-situ*' melting of phosphide. Some of the small phosphides at the cohenite–kamacite interfaces were

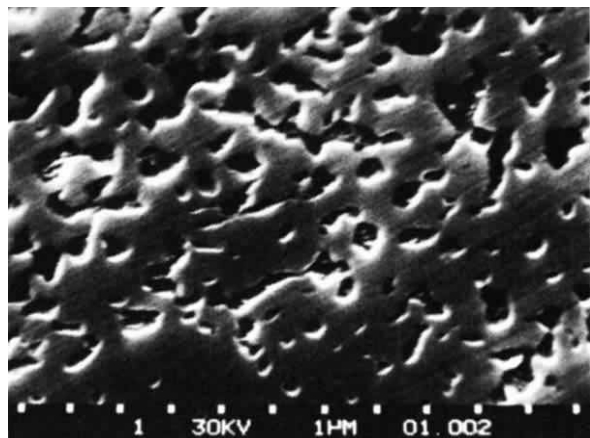


Fig. 3 Scanning electron micrograph of cloudy area in Fig. 2. Field of view 0.015×0.012 mm.

subject to this combination of effects and underwent incipient melting, with the result that the melt penetrated along shear zones in the carbide either as a wedge or as a more extensive band. In either case the process may be visualised as a combination of incipient melting, together with melt penetration along a zone of internal brecciation in the brittle cohenite. As judged by the extent of penetration this would result in considerable ($>10^{-3}$) dilution of the nickel and phosphorus content of the original phosphide. After these shock events the sample reverted to a residual temperature that was sufficiently high to allow some annealing of the earlier shock structure, as is shown by the visible graphitisation in the cohenite.

Following these observations on specimen H 37.546 we re-examined the 'rim' specimens of Axon and Couper² and found cloudy wedges as rare minor features in the cohenite of their specimens 34.5421; 34.2731 and 34.2737. However, the effect was pronounced in specimen 34.FRI. This latter specimen contained major shear displacements and also considerable quantities of shock melted sulphide which confused the identification of the wedge effect. Fortunately, sulphide is not present in the new specimen H 37.546.

H. J. AXON
W. R. D. COUPER
J. KINDER

*Metallurgy Department,
University of Manchester,
Manchester, UK*

Received 25 March; accepted 19 April 1977.

¹ Heymann, D., Lipschutz, M. E., Nielson, B. & Anders, E. *J. geophys. Res.* **71**, 619, (1966).

² Axon, H. J. & Couper, W. R. D. *Mineral. Mag.* **40**, 827, (1976).

³ Buchwald, V. F. *Handbook of Iron Meteorites* (University of California Press, 1976).

⁴ Brentnall, W. D. & Axon, H. J. *J. Iron Steel Inst.* **200**, 947 (1962).

A possible effect of ice ages on the Earth's magnetic field

ICE ages and reversals of the Earth's magnetic field are two widely different geophysical phenomena; but they may be casually linked by the following mechanism. The moment of inertia of the Earth will be changed by variations in size of polar ice sheets and the resulting redistribution of water mass. To conserve angular momentum the Earth's rotation must therefore change. Calculations on the basis of a simple model then suggest that conditions at the core–mantle boundary may be perturbed in such a way as to affect the generation of the Earth's magnetic field.

Although it is difficult to confirm a connection between climatic and geomagnetic changes, such a relationship could have important consequences in several fields. For example, the relatively well dated palaeomagnetic chronology could be used to increase the resolution of identifying and dating palaeoclimatic episodes; and modelling ways in which the reversal frequency of the Earth's magnetic field might change, or modelling even the mechanism of a field reversal itself, could be constrained by a need to be compatible with changes occurring from variations in the Earth's rotation. Also, this may be a mechanism for using solar energy to drive core motions.

A full analysis of the effect of variations in global ice volume and distribution on the Earth's speed of rotation would require a spherical harmonic approach. Although the formalism exists¹ the calculations do not yet appear in the literature. For simplicity, therefore, estimates of the order of magnitude of quantities involved are found from an idealised model to test if the hypothesis is worth further consideration. A rigid mantle is assumed to be decoupled from the core but initially rotating at the same rate. The atmosphere and ocean are assumed to

rotate with the mantle. The difference in inertia between a uniform layer of water, equivalent to a thin spherical shell, and a polar ice cap composed of the same mass of water circling the globe as an annulus is given by

$$\Delta I = I_s - I_i$$

where $I_s = (\frac{2}{3})Mr^2$ and $I_i = M(r\cos\phi)^2$, M is the mass of water or ice, r is the radius of the Earth and ϕ is the mean latitude of the ice sheet. Assuming that the angular momentum stays constant, then the change in rotational energy is given by $\frac{1}{2}\Delta I\omega^2$ where ω is the angular velocity of the Earth. Taking M as 4.3×10^{19} kg (equivalent to a sea level change of about 100 m), r as 6.4×10^6 m, ϕ as 60° and ω as 7.27×10^{-5} rad s^{-1} then the change in rotational energy is 1.9×10^{24} J. The fractional change in the angular velocity, the length of the day and the moment of inertia is 10^{-5} , in agreement with other estimates². Because in the model the core is decoupled from the mantle there will be a relative velocity of around 2×10^{-3} m s^{-1} at the core-mantle boundary. Complete coupling between core and mantle will change the core energy by about 1.9×10^{23} J, taking the moment of inertia of the core to be a tenth that of the mantle.

Before exploring the implication of these calculations, the effect of isostasy and changes in the Earth's gravitational potential will be discussed. The timescale for ice sheet growth or decay³ and the isostatic response time⁴ are often considered to be of the same order of magnitude, around 2,000 yr. Therefore if the growth or decay of an ice sheet is such that there is a linear change in the moment of inertia over a time T , and if isostatic adjustment occurs as an exponential change of the moment of inertia with a response time equal to T , then the maximum change in the moment of inertia is reduced by e^{-1} . The fractional changes in the length of the day and the speed of rotation of the Earth will be affected in a similar way. Although not contained in the model considered here, it is important to realise that even when an ice sheet has reached isostatic equilibrium there is still a small difference in the moment of inertia compared with the case where there is no ice sheet⁵; previous calculations of the effects of fluctuating ice volumes on the rotation of the Earth have usually assumed full isostatic compensation⁵ and have thus ignored the much larger transient changes. Farrell and Clark¹ have shown that the gravitational attraction of an ice sheet prevents eustatic sea level changes occurring as a uniform layer of water because the resulting ocean surface would not be an equipotential (that is, there is not a constant change in sea level worldwide). The effect is greatest near the ice sheet and only slightly increases the change in the moment of inertia previously calculated. Changes in gravitational and elastic energy are henceforth ignored.

Assuming a timescale of around 2,000 yr for variations in the size of ice sheets and for isostatic relaxation, an upper limit for the rate of average energy dissipation in the core would be 5×10^{12} W. Estimates⁶ of the power requirements for the geodynamo range from 10^9 to 10^{12} W. Therefore a perturbation of the magnetic field cannot be immediately discounted on energetic grounds. A possible core-mantle coupling mechanism for transferring energy to the core is one suggested by Hide⁷ to explain the decade variations in the length of the day, which are similar in magnitude to those arising here. Shallow topographic features at the core-mantle interface allow horizontal stresses to transfer angular momentum between core and mantle, whereas viscous and electromagnetic torques are not strong enough. Supposing that the polarity of the field depends on comparatively minor features of the pattern of core motions, Hide⁷ has suggested that changes in topography or the temperature field on the core-mantle boundary can lead to changes in the frequency of field reversals. The westward drift of the Earth's non-dipole field at around 3×10^{-4} m s^{-1} has been thought to indicate the magnitude of core motions⁷. In the model considered here, this velocity would be reached after 300 yr. Timescales of this order seem to be present in some

features of the Earth's non-dipole magnetic field⁸. Precessional¹ forces are an alternative coupling mechanism. They are thought to be insufficient to drive the geomagnetic dynamo⁶, but may still be large enough to modulate the magnetic field. Precessional torques at the core-mantle boundary would arise from changes in the mantle's principal moments of inertia⁵. Theoretical studies of dynamos generated by motions of a conducting fluid have shown their sensitivity to their boundary conditions⁹. It is therefore plausible that a continuous change in the speed of rotation of the Earth over a sufficiently long time could alter conditions at the core-mantle boundary and perturb the magnetic field or even cause a reversal. A number of mathematical models¹⁰⁻¹³ developed to explain the observed frequency distribution of polarity intervals all require reversals to occur by random variations in the orientation or distribution of subsidiary dipoles. It is therefore implied that the energy needed to trigger a field reversal could be much less than that needed to sustain dynamo action.

Evidence for a correlation between glacial periods and changes in the magnetic field is hard to evaluate because of the difficulty in accurately identifying and dating variations in both parameters. It is interesting though to speculate on a number of observed coincidences between changes in the magnetic field and in various other phenomena which might be explained by the hypothesis presented here. Wollin *et al.*¹⁴ have found from an examination of some deep-sea sedimentary cores that changes in intensity of the magnetic field could be correlated over the last 2 Myr with climatic changes represented both by variations in the population of foraminifera and by oxygen isotope changes thought to indicate fluctuations in ice volume. Kent and Opdyke¹⁵ have also found from spectral analysis of deep-sea sedimentary cores an apparent correlation between variations in intensity of the magnetic field and changes in the earth's obliquity. They suggest that the precessional force on the core will vary with the obliquity, and that their results reflect the modulation of a precessionally-driven dynamo by changes in the obliquity. Climatic changes affecting the precession of the earth could thus give rise to geomagnetic reversals. Hays¹⁶ has shown that past extinctions of faunal species have occurred nearly simultaneously with reversals of the magnetic field; climatic change associated with glacial activity could increase the environmental stress on the species leading to their destruction. Cox¹⁷ has shown that the reversal frequency of the magnetic field increased about 75 Myr ago and again between 50 and 35 Myr ago. The South Pole has been positioned over the Antarctic continent for about the last 80 Myr (ref. 18), Australasia parted from Antarctica about 50 Myr ago and the Drake Passage joining Antarctica to South America opened about 30 Myr ago¹⁹. This growing thermal isolation of Antarctica led to increasing glacial activity in the Southern Hemisphere²⁰. Harrison and Ramirez²¹ modelled the apparently limited extent of the Laschamp event by combining an axial geocentric dipole with another dipole near the core-mantle boundary beneath Europe; deglaciation of Northern Europe was contemporaneous with this magnetic event, and may be causally associated with it by affecting conditions at the core-mantle boundary. It may be significant that the time taken for the field to complete a reversal is of the order of a few thousand years, the same as the transient non-equilibrium behaviour of the earth at the beginning and end of ice ages.

I thank Professor J. A. Jacobs, Dr R. Hide and Dr D. Gubbins for their critical and helpful comments on some of the ideas expressed here.

CHRISTOPHER S. M. DOAKE

British Antarctic Survey,
Madingley Road,
Cambridge, UK

Received 22 December 1976; accepted 19 April 1977.

¹ Farrell, W. E. & Clark, J. A. *Geophys. J. R. astr. Soc.* **46**, 647-667 (1976).

² Young, A. *Mon. Not. R. astr. Soc., Geophys. suppl.* **6**, 453-457 (1953).

³ Weertman, J. *J. Glac.* **5**, 145-158 (1964).

- 4 O'Connell, R. J. *Geophys. J. R. astr. Soc.* **23**, 299–327 (1971).
 5 Munk, W. M. & MacDonald, G. J. F. *The Rotation of the Earth* (University Press, Cambridge, 1960).
 6 Rochester, M. G., Jacobs, J. A., Smylie, D. E. & Chang, K. F. *Geophys. J. R. astr. Soc.* **43**, 661–678 (1975).
 7 Hide, R. Q. *Jl. R. met. Soc.* **96**, 579–590 (1970).
 8 McElhinney, M. W. *Palaeomagnetism and Plate Tectonics*, (University Press, Cambridge, 1973).
 9 Bullard, E. & Gubbins, D. *Geophys. Astrophys. Fluid dynamics* **8**, 43–56 (1977).
 10 Cox, A. J. *Geophys. Res.* **73**, 3247–3260 (1968).
 11 Nagata, T. J. *Geomag. Geoelec.* **21**, 701–704 (1969).
 12 Parker, E. N. *Astrophys. J.* **158**, 815–827 (1969).
 13 Kono, M. *Phys. Earth Planet. Int.* **5**, 140–150 (1972).
 14 Wollin, G., Ericson, D. B. & Wollin, J. *Colloques Internationaux du C.N.R.S. No. 219*, 273–288 (1974).
 15 Kent, D. V. & Opdyke, N. D. *Nature* **266**, 156–159 (1977).
 16 Hays, J. D. *Bull. geol. Soc. Am.* **82**, 2433–2444 (1971).
 17 Cox, A. *Rev. Geophys. Space Phys.* **13**, 35–51 (1975).
 18 Smith, A. G. & Briden, J. C. *Mesozoic and Cenozoic Palaeocontinental Maps*, (Cambridge University Press, 1977).
 19 Barker, P. F. & Griffiths, D. H. *Phil. Trans. R. Soc.* **279B**, 143–159 (1977).
 20 Shackleton, N. J. & Kennett, J. P. in *Initial Reports of the Deep Sea Drilling Project* (eds Kennett, J. P. et al.) **29**, 743–755 (U.S. Government Printing Office, Washington, 1975).
 21 Harrison, C. G. A. & Ramirez, E. J. *Geomag. Geoelec.* **27**, 139–151 (1975).

The major stratospheric warming of 1976–77

MANY mid-winter warmings of the polar stratosphere have been observed in the last 25 years by means of radiosonde and rocket soundings and, recently, by satellites¹. Major warmings involve a reversal of the normal winter temperature gradient and zonal wind component in the stratosphere between middle and high latitudes. (In normal winter conditions temperatures decrease polewards and winds are westerly). We report here on the behaviour of the temperature and wind fields observed at various levels during a major warming and the indication that, in this case, similar changes occurred in both the troposphere and the stratosphere.

Fig. 1 Mean temperature ($\bar{T}$) over the cap north of 50°N plotted at two-day intervals. Climatological values of monthly mean temperatures, plotted at the mid-point of each month were derived from various publications: +, Crutcher²; ▲, Hanevskaja³; ■, Labitzke *et al.*⁶.

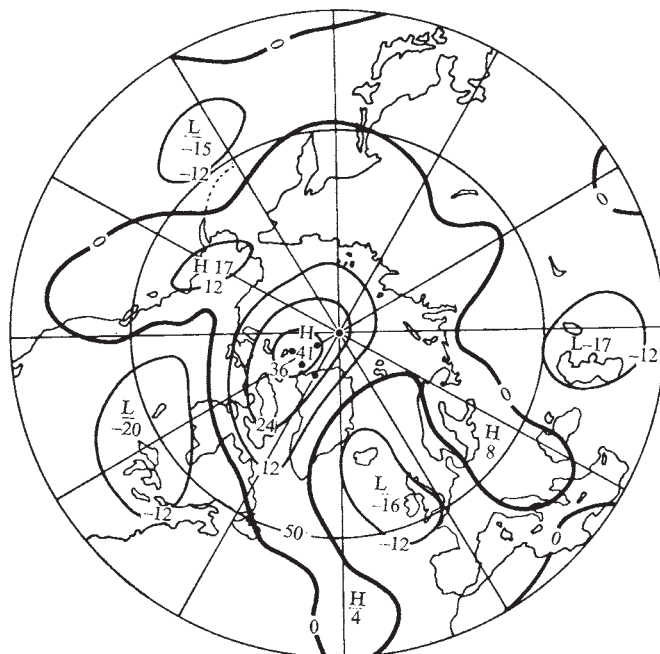
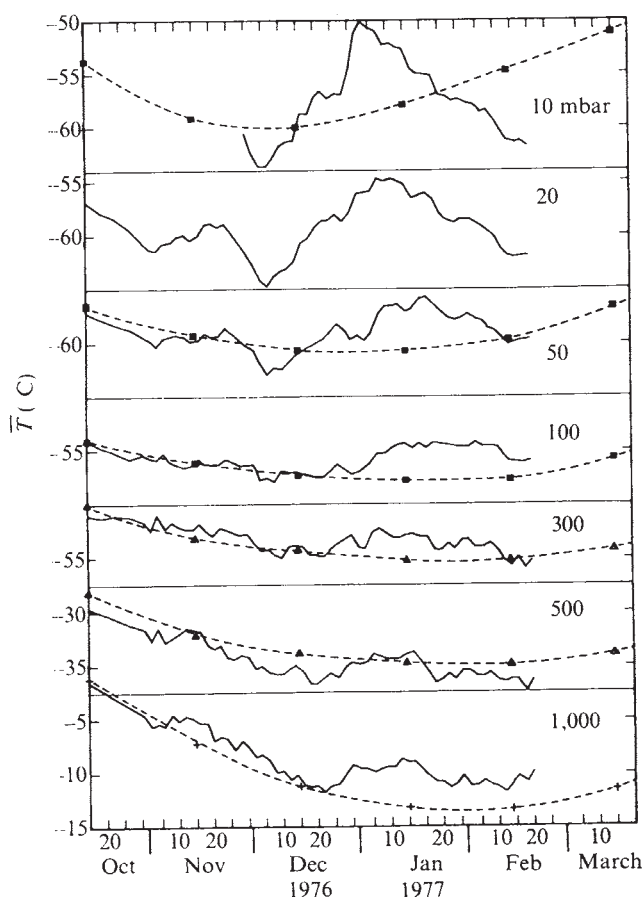


Fig. 2 1,000–500 mbar thickness anomalies (from the 1951–1970 average) for pentad 3, 1977 (11–15 January). Values are shown in geopotential decametres. ●, Grid points at which the anomaly is at least 4σ .

A stratospheric warming which began in December 1976 developed into a major event in January 1977, with zonal mean values of both temperature and geopotential height increasing polewards from 60°N at the 10 and 20 mbar levels. A full analysis of the energetics involved is not yet available (Labitzke *et al.*² have given a synoptic description of the event), but certain unusual aspects of atmospheric behaviour have become apparent from a study of objective analyses for the period.

The Meteorological Office produces twice-daily objective analyses of geopotential height fields at standard pressure levels for the Northern Hemisphere north of about 15°N and, from these analyses, fields of temperature and wind are inferred. We have processed the 00 GMT analyses on alternate days from late 1976, at seven levels (1,000, 500, 300, 100, 50, 20 and 10 mbar) to obtain, at each level, areal mean values of temperature and zonal wind component over the cap north of 50°N.

Variations of temperature during the present winter may be compared with climatological values (Fig. 1). Differences in the treatment of radiosonde data by individual organisations make it difficult to compare absolute values from different sources so the climatological values are included only to demonstrate the average shape of the temperature curves, with the tropospheric minimum occurring in late January or early February. The earlier climatological minimum at 10 mbar probably results from the occurrence, during the five-year period from which the stratospheric mean values were produced, of warmings which affected temperatures from late December onwards.

Two stratospheric warming periods are evident in Fig. 1, the first beginning in early November (during which derived temperatures were not available at 10 mbar) and the second beginning on 6 December and reaching a maximum around 3 January at 10 mbar. During the course of this second stratospheric disturbance, tropospheric temperatures also rose between mid-December and mid-January by some 2 to 3 °C. The anomalies of 1,000–500 mbar thickness from the 1951–70 average for the pentad 11–15 January 1977 show (Fig. 2) predominantly positive values north of 50°N. This chart contains five grid points with anomalies of 4σ

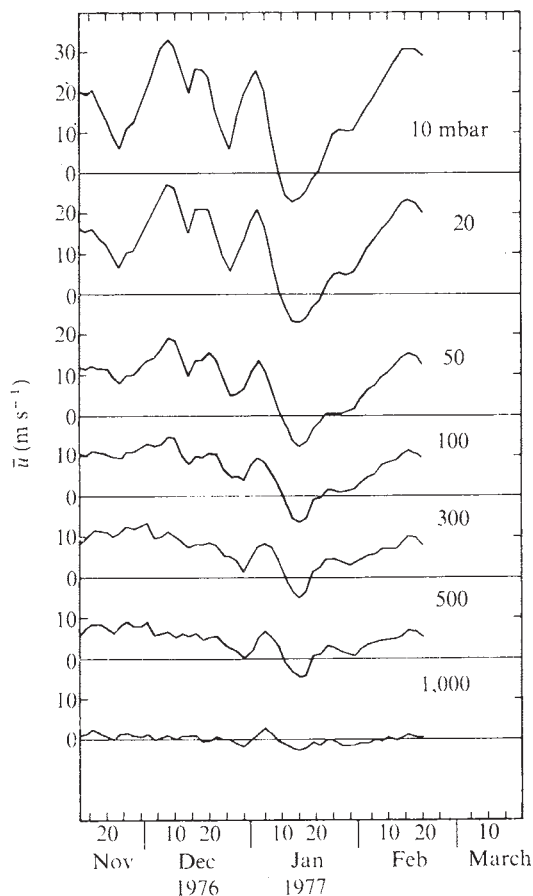


Fig. 3 Mean zonal wind component ($\bar{u}$) over the cap north of 50°N plotted at two-day intervals, (westerly winds are positive). Allowance has been made for surface friction in calculating the winds at 1,000 mbar.

or more where σ denotes the standard deviation for that grid point derived from the 20 values for the corresponding pentad in the years 1951–70; they are all positive and are centred at 80°N, 90°W. The chart for the following 5-d period contains four grid points having similar anomalies; these are centred at 80°N, 145°E. Examination of corresponding thickness anomalies from 50°N to the pole for the previous six winters revealed that only at 21 grid points (of a possible 29,232) did the anomaly reach 4σ . The occurrence of nine extreme values in one 10-d period of mid-January 1977 is in marked contrast to this recent average of three or four per winter: this uncommon tropospheric warming during a stratospheric warming period represents a situation of obvious interest. On the other hand, the apparent lag between the start of the warming at the upper levels on 6 December and the warming in the lower troposphere (about 22 December) should not be interpreted as an indication that the tropospheric warming is caused by the stratospheric disturbance; the determination of any causal relationship between the warmings must await a full analysis of the energetics involved.

The mean zonal component north of 50°N ($\bar{u}$) 10 mbar reversed between 9 and 10 January (Fig. 3). This was associated with a reversal of the meridional gradient of zonal mean temperature in the stratosphere. However, the magnitude of the reversed temperature gradient and the corresponding rate of decrease of $\bar{u}$ with height were small at this stage (and indeed remained so in comparison with a previous major warming in 1967–68 (ref. 2)). The 10-mbar wind reversal was achieved largely because $\bar{u}$ in

the troposphere had also decreased; by 11 January easterlies were present at all levels.

The vertical continuity, so evident during the easterly period, was, in fact, present from late December onwards. A degree of continuity was also present during the minor warming in November minimum values of $\bar{u}$ (though still positive) occurred at all levels at approximately the same time (22–24 November). These results are consistent with the findings of Julian and Labitzke³ who provided evidence for an association between stratospheric warming and a reduction of the 500 mbar zonal wind component in the region 50°N–70°N (used as an indicator of 'blocking'). It may well be that the atmospheric behaviour observed during December and January represents an extreme example of this association.

The synoptic situation associated with the anomalously high polar temperatures and easterly winds of 15 January was dominated by a geopotential high near the North Pole at all levels from the surface up to at least 10 mbar. Tropospheric charts for the easterly period thus have much of the appearance of the typical stratospheric charts during warmings (though also containing features of higher wave numbers not present in the stratosphere). In view of this similarity between these two atmospheric layers it may be unjustifiably restrictive to describe the events as only a stratospheric warming.

B. F. TAYLOR
J. D. PERRY

*Meteorological Office,
Bracknell, Berkshire, UK*

Received 25 March; accepted 14 April 1977.

¹ Barnett, J. J. *et al.* *Nature* 230, 47–48 (1971).

² Labitzke, K. B. *et al.* *Beilage zur Berliner Wetterkarte der Freien Universität Berlin* 27.1.1977.

³ Julian, P. R. & Labitzke, K. B. *J. Atmos. Sci.* 22, 597–610 (1965).

⁴ Crutcher, H. L. *Selected Meridional Cross Sections of Heights, Temperatures and Dew Points of the Northern Hemisphere* NAVAIR 50-1C-59, U.S. Naval Weather Service Command (1971).

⁵ Hanevskaja, I. V. (Ed.) *Atlas of Climatic Characteristics of Temperature, Density and Pressure, Wind and Geopotential in the Troposphere and Lower Stratosphere of the Northern Hemisphere Part II* Moscow (Gidrometeoizdat) (1974).

⁶ Labitzke, K. B. *et al.* *Met. Abh.* Band 100/Heft 4 (1972).

Zonal winds associated with the dry period 1975–76 in England and Wales

THE low rainfall in England and Wales during the period May 1975 to August 1976 was discussed by Morris and Ratchiffe¹ and was shown to be associated with a progressive shift northwards in the position of the mean jetstream over the north-east Atlantic during the three successive years 1974–76. We report here that marked circulation changes in the 500-mbar (5.5 km) flow during the dry period occurred both in the vicinity of the British Isles and over the central Pacific. The flow is compared with an analysis of 5-d mean charts exhibiting blocking of the 500-mbar flow in the vicinity of the British Isles and it is suggested that the anomalous displacement of the jetstream during the dry period may have been associated with an eastward surge in the subtropical jet off south-east Asia.

The actual position of the maximum wind zones around the northern hemisphere varies from season to season but the mean position is conveniently shown by the mean geostrophic zonal west-east component at 500 mbar for the average period 1951–70 (Fig. 1). A northwards displacement of the jetstream occurs not infrequently when a blocking anticyclone is centred in the vicinity of the British Isles, with the shift usually lasting for 5–15 d. The mean zonal flow was studied on occasions when 5-d mean pressure anomaly

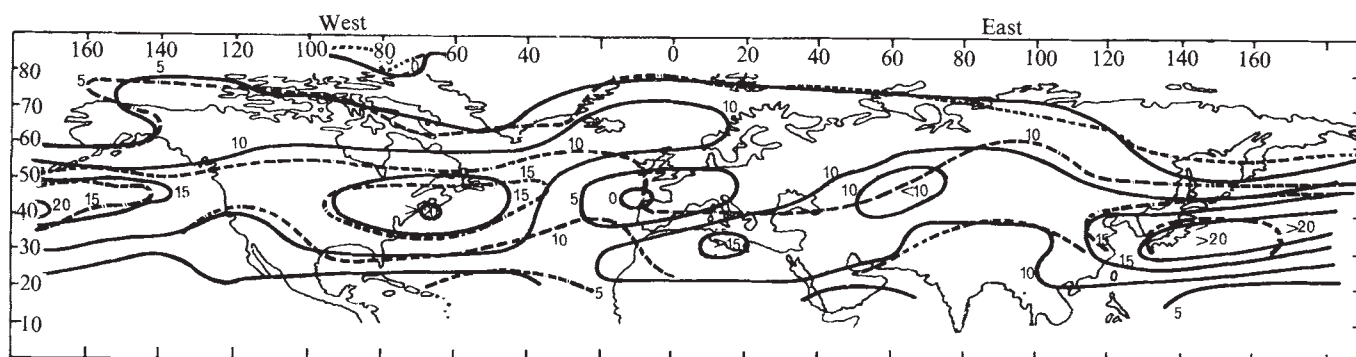


Fig. 1 Broken line, normal (1951–70) 500-mbar zonal components at 5-m s^{-1} intervals. Solid line, composite 500-mbar zonal components at 5-m s^{-1} intervals associated with a positive pressure anomaly in the vicinity of the British Isles.

charts showed a discrete positive pressure anomaly in the vicinity of the British Isles— $50\text{--}60^\circ\text{N}$, $0\text{--}20^\circ\text{W}$ (Fig. 1). On these occasions the maximum in the zonal flow is displaced to the north of the British Isles, further bifurcation of the flow occurs at 60°E , the maximum wind zone off south-east Asia extends further east than the normal position, and there is an eastwards displacement of the south-east Asian trough. Further analysis of the zonal flow shows an almost synchronous eastward surge in the

Pacific temperatures north of 40°N were colder than usual with a mean negative anomaly of approximately 1°C , while temperatures further south were close to normal. Miles³ has also shown that the Pacific was the only sector where the mean north to south temperature gradient in the atmosphere up to 500 mbar (5.5 km) was above average in the latitude band $35\text{--}55^\circ\text{N}$. Sea temperatures are normally conservative over periods of several months and in view of this it is thought that once an anomalous north to south gradient of

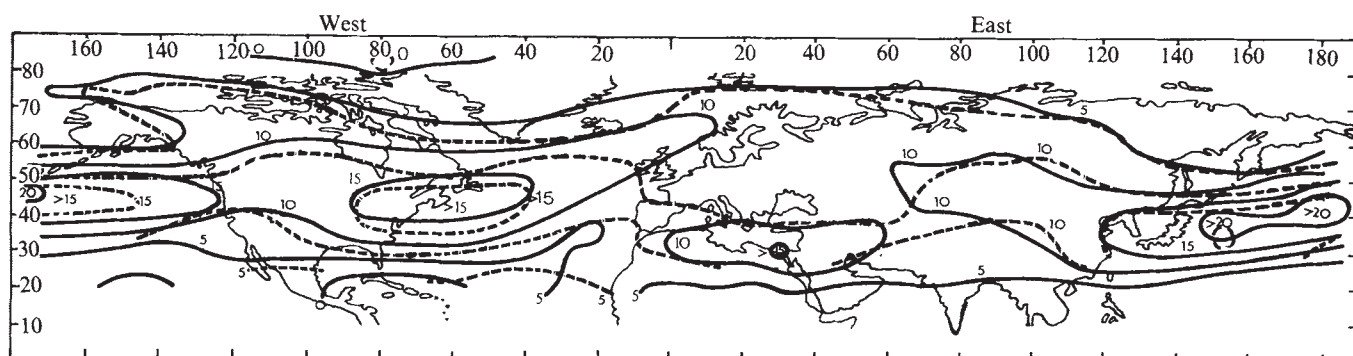


Fig. 2 Solid line, composite 500-mbar zonal components at 5-m s^{-1} intervals for between May 1975 and August 1976. Broken line, pseudo normal (16 months May to August 1951–70) 500-mbar zonal components at 5-m s^{-1} intervals.

500-mbar flow off south-east Asia throughout the year whenever a blocking anticyclone is observed near Britain.

This suggests that the displacement of the jet to the north of the British Isles during the dry period May 1975–August 1976 may have occurred in association with a similar eastwards surge in the subtropical jet off southeast Asia during the same period. Indeed an analysis of the zonal flow at 500 mbar during the dry period in England and Wales which lasted from May 1975 to August 1976 (Fig. 2) shows similar features including extension eastwards of the wind maximum off south-east Asia when compared with the average for the same period. The anomalies in the zonal flow are in the order of plus 4 m s^{-1} both to the north of the British Isles and between 180° and 160°W at 45°N , and are significantly different from the average at the 5% level, using Student's t test. The anomalous eastward displacement of the south-east Asian trough, also observed during the dry period, would by virtue of the hemispherical long wave interdependence result in downstream adjustments within a few days which on the timescales considered here would seem to be synchronous.

During the dry period 1975–76 it has been shown² that

temperature was established in the troposphere, the regime would tend to maintain the enhanced zonal flow over the Pacific and the anomalous eastward position of the south-east Asian trough. It seems, therefore, that the displacement of the jet to the north of the British Isles during the dry period from May 1975 to August 1976 occurred in association with an eastward surge in the sub-tropical jet off south-east Asia during the same period and that the anomalous sea temperature distribution over the Pacific was a factor in maintaining the enhanced flow.

J. D. PERRY

Meteorological Office,
Bracknell,
Berkshire, UK

Received 26 January; accepted 25 March 1977.

¹ Morris, R. M. & Ratcliffe, R. A. S. *Nature* **264**, 4–5 (1976).

² Ratcliffe, R. A. S. *Met. Mag.* **106**, 145–154 (1977).

³ Miles, M. K. *Met. Mag.* **106**, 154–164 (1977).

Weak shock waves in the eruption column

RECENTLY Nairn¹ has made a study of the visible air shock waves which propagate above a volcanic vent in the form of luminous arcs and are closely followed by violent projections of gas and solid ejecta. In ref. 1 it is suggested that this event is caused by a strong explosion within the volcano due to a rapid heating of meteoritic water enclosed in lava pockets. Another type of shock wave formation accompanying volcanic eruption was recorded by one of us (L.D.L.) during an explosive phase of eruption in the Tolbatchik volcanic area (Kamchatka 1975). These waves were smoke and ash which could be observed as optically denser layers rising in the eruption column and probably cannot be attributed to burst phenomena. The present note is based on observations made near the end of the activity of cone II (the middle one in the photographs) when other newly-built pyroclastic cones already showed only weak fumarolic activity.

Over that time (27 August–9 September) the activity of cone II consisted of (1) ejection of a gas stream carrying a great amount of solid particles, (2) jets of ash, lapilli and blocks and (3) a liquid lava emission from the fissure near the cone's foot. Generally the three events took place simultaneously.

The lava temperature near the flow issue is estimated to be about 1,000 °C (ref. 2). The colour of the gas stream at the eruption column base and of blocks and fire jets, taking into account the screen formed by the smoke and ash through which they were observed, suggests they are at temperatures as high as 1,000–1,200 °C. However, the eruption column itself was not luminous and therefore the mean temperature of the gas probably did not exceed 600–400 °C.

Events (1) and (2) were practically continuous but occasional lulls of some 10 min or at most a few hours were recorded. Increases in the violence of gas emission so that even the side of the column exposed to the wind became quite upright—the so-called blastings—were noted also. As a rule the behaviour of the gas stream changed smoothly without any marked preliminary sign but it may be noted that sometimes before the end of a lull in (1) there was an intensification of (2).

In spite of such variations the explosive behaviour might be almost steady for many hours. At these times eruption was accompanied by a roar easily audible within a range of about 10 km and very similar to the noise of a reactive aeroengine fixed in one place. The eruption column was as high as 1 km or more.

At such periods of steady and fairly intense gas ejection one could observe thin optically denser layers cutting across the column. They travelled along the column from the rim of the cone to the cloud with a velocity—relative to the observer—greater than 1 km s⁻¹, most probably of the order of 1.5–3 km s⁻¹. When they met the cloud the layers expanded in diameter and broke up into several parts which in turn rapidly scattered on the lower face of clouds and it appeared to quiver as a result. The visible disintegration of the dense layers was followed by an acoustic shock from above producing in the observer the same sensation as do the air waves generated by aircraft passing through the sound barrier.

The phenomenon described did not occur often, but sometimes several layers followed one another at irregular intervals of some minutes or several tens of minutes.

Emergence of these travelling layers did not correlate with any appreciable changes in the regime of events (1) and/or (2) and was not preceded or followed by distinguishable underground bursts or shots. This behaviour contrasts with the strong atmospheric shock waves discussed in ref. 1: a weak shock wave travelling along the eruption column is unlikely to be connected with explosions within a volcano.

The nature of the explosions in cone II suggests an analogy with a nozzle through which the gas is discharged from the



Fig. 1 *a, b*, Layers of different densities. Photographs showing layers of different densities in the eruption column. Note that the high velocity of layer motion makes their images indistinct. (Photographs by L. D. Livshits).

Earth's interior to the atmosphere. The gas stream may be supersonic. If we assume that the rate of gas flow is sufficiently great that the flow is steady, we have a situation in which dense layers will form. For the estimation of flow rate the Bernoulli equation may be used

$$\frac{1}{2}v_0^2 + C_p T_0 = \frac{1}{2}v^2 + C_p T$$

Suppose that when the gas velocity v_0 is zero the gas temperature T_0 is equal to that of the surrounding rocks (= 1,000–1,200 °C) and that the specific heat $C_p = 1 \text{ J g}^{-1} \text{ K}^{-1}$. We

obtain the result that when cooling to 600–400 °C (the likely temperature of gas in the eruption column) the flow rate may be as great as 1,100 m s⁻¹. For a rough estimate of the sound velocity C in the gas at 600 °C the expression $C \approx (T/T_A)^{1/2}$ gives only about 600 m s⁻¹, where the subscript a refers to the atmospheric conditions. This indicates that the gas flow may indeed be supersonic.

The pressure on the nozzle exit is expressed by the equation

$$p = p_0 \left(1 - \frac{\gamma - 1}{2} \frac{v^2}{C_0^2} \right)^{\frac{\gamma}{\gamma - 1}}$$

where p_0 , C_0 and p , C are the pressure and sound velocity of gas at the nozzle entrance and exit respectively.

If the jet pressure is a positive one ($p > p_a$) the jet stream will expand laterally (in the expansion wave). If the jet pressure is less than atmospheric ($p < p_a$), a pressure discontinuity of the shock type is initiated. In this shock wave (which is always a weak one) the value of the pressure is increased to p_a . To evaluate the gas outlet velocity v corresponding to $p = p_a$ we can use the expression

$$v^* = C_0 \left(\frac{2}{\gamma - 1} \right)^{1/2} \left[1 - \left(\frac{p_a}{p_0} \right)^{(\gamma - 1)/\gamma} \right]^{1/2} \approx C_0 \left(\frac{2}{\gamma - 1} \right)^{1/2}$$

By putting $(p_a/p_0)^{(\gamma - 1)/\gamma} \ll 1$, $C_0 \approx C_a(T_0/T)^{1/2}$, $\gamma = 1.4$ we obtain a value of v^* of approximately 1600 m s⁻¹. The value of v^* determines the position of the shock wave in reference to the nozzle exit: when the gas velocity v is greater than v^* the pressure in the channel is reduced and the shock wave moves upstream toward the nozzle entry; when v is less than v^* , the shock wave separates and moves in downstream direction. In 'normal' conditions such a surge would diverge and disintegrate right after separation. But the divergence of the weak shock wave under consideration here is oppressed by acting as a waveguide—the hot gas column is surrounded by cooler atmospheric walls of greater acoustic stiffness (the product of density and velocity of sound). So after separation the shock wave travels along the eruption column with a velocity equal to the velocity of sound in a hot gas. The velocity of the shock wave relative to the observer would be ~ 2 km s⁻¹ as was visually estimated.

In the cloud the acoustic stiffness of the column walls changes and the shock wave is able to diverge. The process is enhanced by reflections of the shock wave especially downwards against the supersonic gas flow. These create a zone of superpressure at the level of lower edge of cloud. The expansion of superpressured gas causes the visible effect of local motion in the lower part of the cloud and generates the sonic bang mentioned above.

The model presented here allows interpretation of phenomenon described in terms of the gas dynamics of the jetstream.

For strong shock waves the column of hot gas and tephra did not serve as a waveguide. Therefore the flashing arcs would pass in the atmosphere irrespective of presence of eruption column (as has been drawn by Perret³) or in its absence, as one can see on photographs in Nairn's paper¹.

L. D. Livshits thanks Dr A. J. Farberov for the opportunity to take part in the Tolbatchik research expedition.

L. D. LIVSHITS
L. G. BOLKHOVITINOV

O. Yu. Shmidt Institute of Physics of the Earth,
Academy of Sciences of the USSR,
123242, Moscow, USSR

Singlet oxygen in natural waters

MANY studies have shown that singlet molecular oxygen can oxidise a variety of organic substances^{1–7}. These studies have included biologically important compounds present in the aquatic environment such as amino acids^{4,7} and pollutants like polycyclic aromatic hydrocarbons⁵ and pesticides⁶. No data, however, have been obtained to demonstrate that reactions involving singlet oxygen (often called oxygenations) are sufficiently rapid to be significant in natural waters. The most likely mechanism for oxygenation in the environment was originally proposed by Kautsky⁸ by which light energy absorbed by a sensitizer is transferred to ground-state oxygen to form singlet oxygen, which in turn reacts with the organic substance, or 'acceptor' (A), to form a peroxide. Here we present evidence that rapid photochemical generation of singlet oxygen occurs in inland and coastal water bodies of the south-eastern United States.

Samples from selected water bodies were collected in pre-cleaned 1-l bottles with aluminium foil-lined caps. In all cases, water samples were obtained from the top half-metre of the water body and refrigerated at 5 °C before use. Very turbid samples from the Mississippi and a pond in Athens, Georgia, were centrifuged before use to facilitate analysis by liquid chromatography. All other samples were used with no pre-treatment. The samples exhibited a wide range of pH and total organic carbon. (Table 1).

The singlet oxygen, a short-lived species in water⁹, was chemically trapped by 2,5-dimethylfuran (DMF) that was added to the water samples. DMF was chosen because it is highly reactive towards singlet oxygen and its photo-oxygenation products have been well characterised¹⁰. Because DMF has a high tendency to volatilise from water, kinetic studies were conducted in cells equipped with gas-tight Teflon stopcocks. We analysed for DMF and the photoproducts by high-pressure liquid chromatography (HPLC) using 40% methanol-water as the mobile phase and ODS as the stationary phase.

Detailed product studies were conducted on the brown-coloured water from the Okefenokee Swamp. A solution of DMF in air-saturated swamp water was exposed to sunlight for an hour, then the products were collected on prewashed XAD-4 resin¹¹ and isolated by preparative HPLC. The major product (85% yield) was identified as *cis*-1,2-diacetylene by comparison of its mass and NMR spectra with spectra of an authentic sample (mass spectrum: m/e (rel. intensity, 112 (7), 96 (59), 69 (29), 43 (100); NMR (CDCl₃), δ 2.30 (singlet, 6H), δ 6.36 (singlet, 2H)). Two minor products were formed, one of which was identified as *trans*-1,2-diacetylene¹². Foote and his co-workers¹⁰ have shown that DMF reacts with singlet oxygen to form a peroxide which decomposes to diacetylene in water. HPLC analysis indicated that the same products were formed when solutions of DMF in the other water samples were exposed to sunlight. The DMF did not react in dark controls or when exposed to sunlight in distilled water.

To demonstrate further that singlet oxygen was an intermediate in these reactions, the effects of DABCO (1,4-diazabicyclo[2,2,2]octane) and deuterium oxide were studied (Fig. 1). The photo-oxygenations were strongly inhibited by DABCO, an efficient quencher of singlet oxygen¹³. On the other hand, and again consistent with the singlet oxygen mechanism, the reactions were more rapid in air-saturated 1:1 mixtures of deuterium oxide–natural water than in 1:1 mixtures of distilled water–natural water. The acceleration is attributed to an increase in the lifetime of singlet oxygen in the presence of deuterium oxide⁹. DABCO and deuterium oxide affected the reaction in Okefenokee Swamp water in nearly the same way as they affected photo-oxygenation of DMF using rose bengal, a known singlet oxygen sensitizer¹⁴ (Fig. 1).

When the concentration of acceptor is low, it has little in-

Received 24 January; accepted 29 March 1977.

¹ Nairn, I. A. *Nature* **259**, 190–192 (1976).

² Tchirkov, A. M. *Priroda* **7**, 80–93 (1976) (in Russian).

³ Green, J. & Short, N. M. *Volcanic Landforms and Surface Features*, plate 9B, (Springer-Verlag, New York, 1971).

Table 1 Kinetic parameters for photochemical generation of singlet oxygen in natural waters from the south-eastern United States

Water body	pH	Total organic carbon (mg l ⁻¹)	F_{366}^*	$(Y_s)_{366}^\dagger$	Sunlight‡ $t_{1/2}$ (min)	$[O_2^*] \times 10^{13}$ M§
Okefenokee Swamp, Georgia	4.1	24	0.389	0.056	13	22
Aucilla River, Lamont, Florida	6.0	24	0.446	0.026	16	18
Econfina River near Perry, Florida	4.2	41	0.658	0.032	15	19
Fenholloway River, Foley, Florida	7.7	77	0.941	0.014	17	17
Wakulla River near Wakulla Springs, Florida	8.6	6	0.027	0.060	150	2
St Marks River, St Marks, Florida	8.7	9	0.165	0.030	51	6
Gulf of Mexico, Shell Point, Florida	8.5	6	0.050	0.060	100	3
Gulf of Mexico, Live Oak Island, Florida	8.3	6	0.060	0.052	—	—
Puddle in peanut field, Sylvester, Georgia	6.6	12	0.113	0.065	33	10
Mississippi River, Baton Rouge, Louisiana	8.3	14	0.060	0.093	60	5
Hickory Hills Pond, Athens, Georgia	7.2	4	0.041	0.019	—	—

*Fraction of 366-nm light absorbed in 1.00 cm.

†Quantum efficiency for singlet oxygen formation at 366 nm.

‡Midday half life for photo-oxygenation of 2,5-dimethylfuran under December sunlight.

§Steady-state concentration of singlet oxygen.

fluence on the lifetime of singlet oxygen, τ , and the rate expression for disappearance of acceptor is

$$-\frac{d[A]}{dt} = \frac{I_a Y_s}{\beta} [A] \quad (1)$$

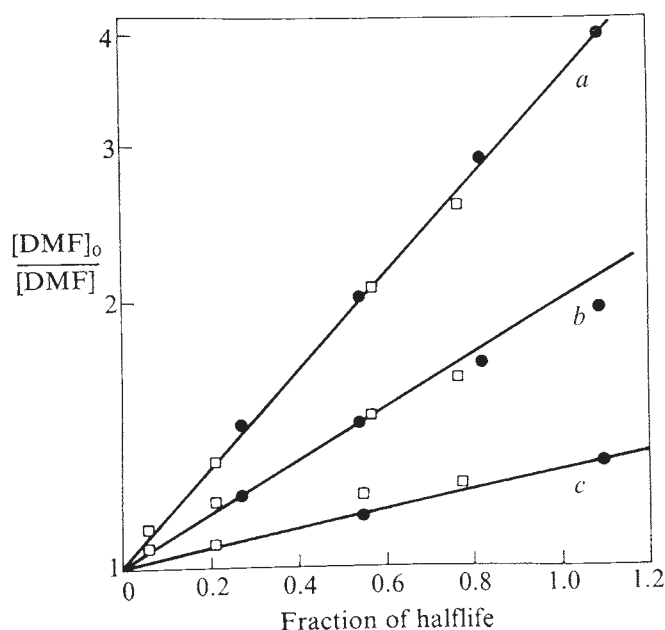
where I_a is the rate of light absorption and Y_s is the quantum efficiency for singlet oxygen generation¹⁵. The constant, β , equals $1/(k_A \tau)$ where k_A is the rate constant for reaction between

singlet oxygen and acceptor and τ is the lifetime of singlet oxygen^{1,9}. Data in Fig. 1 indicate that τ is about the same in distilled and swamp water. To determine values of Y_s for the water samples, air-saturated solutions of 10^{-4} M DMF in natural water and potassium ferrioxalate actinometers¹⁶ were exposed simultaneously to monochromatic light (366 nm) in a 'merry-go-round' apparatus¹⁷. Values of Y_s were computed from slopes of first-order plots, assuming that β for DMF is $1.2 \times 10^{-3} \text{ mol l}^{-1}$ in all the water samples⁹. These values ranged from a low of 0.014 to a high of 0.093 with an average of 0.043 ± 0.019 in our water samples (Table 1). In view of the reported complexity of the substances in natural waters¹⁸, the observed variation in quantum efficiencies was remarkably small.

Of particular environmental interest are the steady-state concentrations of singlet oxygen, $[O_2^*]$, that are photochemically generated by the action of sunlight. Near-surface concentrations of O_2^* were estimated by exposing 1-cm thick solutions of DMF (10^{-4} M) in the natural waters to sunlight in Athens, Georgia, (latitude 34°N) during December. Steady-state concentrations were assumed to equal $0.693 (k_A t_1)^{-1}$, where k_A is $4 \times 10^8 \text{ l mol}^{-1} \text{ s}^{-1}$, the rate constant for the reaction of DMF with singlet oxygen⁹, and t_1 is the first-order half life observed in the sunlight experiments (Table 1). Changes in $[O_2^*]$ were closely paralleled by changes in the rates of O_2^* generation at 366 nm, as reflected by the product, $F_{366} (Y_s)_{366}$ (Table 1). Taking the underwater solar irradiance values reported by Zepp and Cline¹⁹, we estimated that midsummer values are about three times higher than these midwinter values. The highest rates of singlet oxygen formation occurred in water from the highly coloured rivers of north Florida and the Okefenokee Swamp (first four in Table 1). These water bodies contain high concentrations of organics, most of which is fulvic acid, a complex humic substance derived from decayed plants¹⁹. Because most inland and coastal water bodies contain fulvic acid, it is reasonable to infer that photochemical generation of singlet oxygen is a widespread phenomenon in the aquatic environment.

The kinetic parameters in Table 1 can be used to estimate approximate half-lives for photo-oxygenation of dissolved organics in the aquatic environment. Using reported^{4,5} values of k_A , the following half-lives were estimated for photosensitised oxygenation of selected polycyclic aromatics and amino acids in the Okefenokee Swamp water: 7,12-dimethylbenz(a)anthracene, 6 h; naphthacene, 7 h; pentacene, 0.02 h; histidine, 2 h; tryptophan, 2 h; methionine, 3 h. The pesticide, *cis*-resmethrin²⁰, a furan derivative, must react at about the same

Fig. 1 Effect of DABCO and deuterium oxide on photo-oxygenation of 2,5-dimethylfuran (DMF) in Okefenokee Swamp water and in distilled water containing rose bengal; initial DMF concentration $[DMF]_0$, 1.0×10^{-4} M. Duration of light exposure expressed as fraction of half life compared with systems with no added DABCO or deuterium oxide (line *b*). *a*, 1:1 mixture of swamp water or 10^{-4} M aqueous rose bengal with deuterium oxide; *b*, 1:1 mixture of swamp water or 10^{-4} M aqueous rose bengal with distilled water; *c*, 1:1 mixture of swamp water or 10^{-4} M aqueous rose bengal with 0.019 M aqueous DABCO. Light source was a 450-W high-pressure mercury lamp; light was filtered through Corning 7-83 glass filters to isolate 366-nm line. ●, Rose bengal; □ Okefenokee swamp.



rate as DMF. These half lives apply only to organics dissolved in the water. In water containing an appreciable amount of suspended sediments, extremely hydrophobic organics, such as polycyclic aromatics, exist in both the dissolved and sorbed state²¹. Nonetheless, the computed half lives do serve to indicate that singlet oxygen is likely to be an important intermediate in the transformation of certain pollutants and biologically important substances in lakes and rivers. Also, it is possible that the peroxides which form in singlet oxygen reactions may initiate the free radical oxidation of aquatic pollutants.

Finally, these findings suggest that singlet oxygen may be involved in the well known sunlight-induced fading of the colour in natural waters¹⁸. In preliminary studies of this phenomenon, we found that the Okefenokee Swamp water underwent considerable fading when exposed to sunlight for one full day. This fading did not occur when oxygen was removed from the water by degassing before sunlight exposure.

We acknowledge the assistance of Drs R. H. Cox and C. W. Pape in synthesising reaction products.

RICHARD G. ZEPP
N. LEE WOLFE
G. L. BAUGHMAN
REGINALD C. HOLLIS

Environmental Research Laboratory,
US Environmental Protection Agency,
College Station Road,
Athens, Georgia 30601

Received 7 February; accepted 12 April 1977.

- 1 Foote, C. S. *Acc. Chem. Res.* **1**, 104-110 (1968).
- 2 Gollnick, K. *Adv. Photochem.* **6**, 1-122 (1968).
- 3 Kearns, D. R. *Chem. Rev.* **71**, 395-427 (1971).
- 4 Nilsson, R., Merkel, P. B. & Kearns, D. R. *Photochem. Photobiol.* **16**, 117-124 (1972).
- 5 Stevens, B., Perez, S. R. & Ors, J. A. *J. Am. chem. Soc.* **96**, 6846-6850 (1974).
- 6 Wolfe, N. L., Zepp, R. G., Baughman, G. L., Fincher, R. C. & Gordon, J. A. *Chemical and Photochemical Transformation of Selected Pesticides in Aquatic Systems*, (US Environmental Protection Agency, Athens, GA, rep. no. EPA-600/3-76-067, 1976).
- 7 Foote, C. S. in *Free Radicals in Biology, II* (edit. by Pryor, W.), 85-133 (Academic, New York, 1976).
- 8 Kautsky, H. *Trans. Farad. Soc.* **35**, 216-219 (1939).
- 9 Merkel, P. B. & Kearns, D. R. *J. Am. chem. Soc.* **94**, 7244-7253 (1972).
- 10 Foote, C. S. *et al. Tetrahedron* **23**, 2583-2599 (1967).
- 11 Junk, G. A. *et al. J. Chromatogr.* **99**, 745-762 (1974).
- 12 Armstrong, K. F. & Robinson, R. J. *chem. Soc.* 1650 (1934).
- 13 Oannes, C. & Wilson, T. J. *Am. chem. Soc.* **90**, 6528 (1968).
- 14 Gollnick, K., Franken, T., Schade, G. & Dörhöfer, G. *Ann. N. Y. Acad. Sci.* **171**, 89-107 (1970).
- 15 Young, R. H., Wehrly, K. & Martin, R. L. *J. Am. chem. Soc.* **93**, 5774-5779 (1971).
- 16 Hatchard, C. G. & Parker, C. A. *Proc. R. Soc. A* **235**, 518-536 (1956).
- 17 Moses, F. G., Liu, R. S. H. & Monroe, B. M. *Molec. Photochem.* **1**, 245-250 (1969).
- 18 Gjessing, E. T. *Physical and Chemical Characteristics of Aquatic Humus*, 1-84 (Ann Arbor Science, Ann Arbor, 1976).
- 19 Zepp, R. G. & Cline, D. M. *Environ. Sci. Technol.* **11**, 359-366 (1977).
- 20 Ueda, K., Gaughan, L. C. & Casida, J. E. *J. Agric. Fd. Chem.* **22**, 212-220 (1974).
- 21 Andelman, J. B. & Snodgrass, J. E. in *Critical Reviews in Environmental Control* **5**, 69-83 (Chemical Rubber Company, Cleveland, 1974).

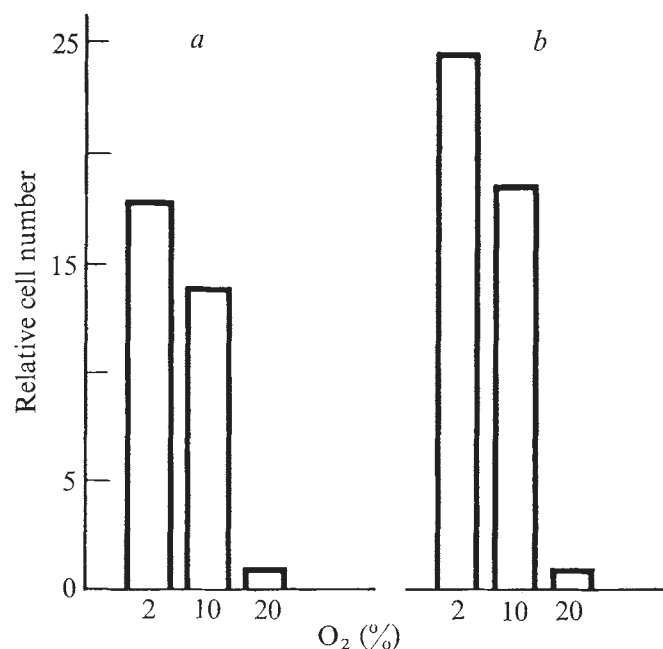


Fig. 1 Effect of oxygen concentration on the growth of IMR-90 (a) and WI-38 (b) human diploid cells. Cells were grown in Eagle's Basal Medium with Hanks' salts (HBME, Pacific Biologicals) to which was added 50 $\mu\text{g ml}^{-1}$ aureomycin (Lederle), 2.8×10^{-2} M *N*-2-hydroxyethylpiperazine-*N*'-2-ethanesulphonic acid (HEPES, Sigma) and 10% (v/v) non-inactivated foetal calf serum (GIBCO). Young WI-38 cells at PDL 23 and IMR-90 cells at PDL 19 were seeded in T-25 flasks at about 2,000 cells per flask and kept in a desiccator at 37 °C at the indicated oxygen concentration for 10 d. Cell counts performed in triplicate for each oxygen concentration are given as relative values based on a value of 1 for the 20% oxygen cell counts: for IMR-90 this was 18×10^3 ; for WI-38 this was 28×10^3 .

be appreciably enhanced by more exact control of environmental factors.

We have studied the effect of oxygen concentration on growth and lifespan, primarily using WI-38 cells which are generally accepted to exhibit a limited *in vitro* lifespan¹. But the normal human lung strain IMR-90 has also received study more recently. Therefore, we felt it important to carry out parallel experiments using both types of cells. Cultures were tested periodically for mycoplasma by the culture method and found to be negative. Cell culture techniques have been described in detail elsewhere^{2,3}. WI-38 and IMR-90 cells were exposed to varying oxygen concentrations in the gas phase over the culture medium, and the effect on growth and lifespan evaluated. The term 'growth' as used in this study is meant as increase in cell number and not as increase in cell size. The population doubling level was used as a rough indication of culture age and is defined as a twofold increase in the number of cells seeded into a vessel².

Individual flasks were seeded with cells in culture medium. For growth studies, cells were seeded at 0.25×10^6 cells per flask and triplicate flasks were counted daily from days 2-7 inclusive of incubation at various oxygen concentrations. For plating experiments, cells were seeded at a low density (2,000 cells per flask) and cells and colonies were counted after 10 d of growth (with no media change) under varying oxygen levels. The flasks were then flushed for 1 min at a flow rate of 1.5 l min^{-1} with various proportions of oxygen in nitrogen with carbon dioxide held constant at 5% at 1 atm and 20 °C. The following combinations of gas were used: 2% O₂, 93% N₂, 5% CO₂; 5% O₂, 90% N₂, 5% CO₂; 10% O₂, 85% N₂, 5% CO₂; 20% O₂ (added as 95%, dry air which contains 19.99% O₂), 5% CO₂; and 50% O₂, 45% N₂ and 5% CO₂. The gassed flasks were placed in

Low oxygen concentration extends the lifespan of cultured human diploid cells

THE pioneering studies of Hayflick¹ showed that normal human diploid cells of the WI-38 strain have a limited lifespan of approximately 50 ± 10 population doublings (PDL) *in vitro*. It is still not known to what extent this phenomenon is the result of the artificiality of the cell culture system: factors such as nutrition and ambient environmental factors may restrict the ability of these cells to express their true genetic potential. To what extent genetic and environmental factors act to constrain the lifespan of human diploid cells in culture is open to experiment. In the present study we have evaluated one important ambient factor of the environment, the oxygen concentration, on the growth and lifespan of two human diploid cell strains, WI-38 and IMR-90. Our results show that growth at oxygen concentrations less than 20% is enhanced and that an extension of about 25% of the lifespan of both cell types was realised by long term cultivation under 10% oxygen. This indicates that the potential for long term growth in culture of normal diploid cells may

250-mm desiccators from which the air was removed by vacuum and replaced with the gas mixture used in flushing the flasks.

In short term growth studies, within a given series of subcultures, we have generally observed that concentrations of oxygen below 20%, particularly between 5 and 10%, speed up the growth rate. When the results of many experiments are averaged, however, the results were usually found not to be statistically valid. If cells are seeded at lower density, however, the effects of lowered oxygen concentration on cell growth are apparent, relative to cells grown at 20% oxygen; cells cultivated at 10 and 2% oxygen show much increased growth rates (Fig. 1). Such results are similar to the increased clonal growth rates of cultured cells of various types reported previously^{4,5}, that less than 20% oxygen concentration enhances the growth rate of mammalian cells cultured either attached to the substratum or in a suspension culture (compare with ref. 5).

Although neither WI-38 nor IMR-90 showed reproducibly greater rates of short term growth, it seemed possible that cumulative effects of lower oxygen concentration might be seen in lifespan studies, particularly since cell growth was always inhibited, even in the short term, at oxygen concentrations greater than 20%.

A typical series of experiments showing the effects of 10, 20 and 50% oxygen on the lifespan of WI-38 cells is shown in Fig. 2. At 50% oxygen, the cells phase out after two subcultivations, whereas the control cultures grown at ambient 20% oxygen exhibit a finite lifespan of 56 population doublings. When grown continuously at 10% oxygen, the cells did not phase out until 68 population doublings had elapsed. Table 1 summarises four series of experiments with

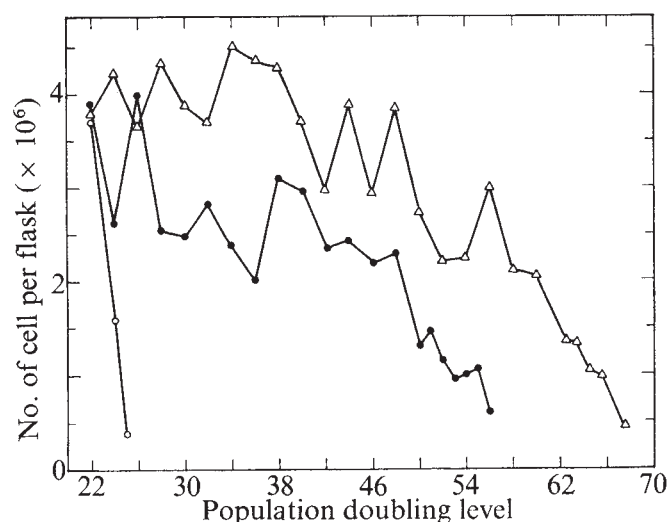


Fig. 2 Effect of oxygen concentration on the lifespan of WI-38 human diploid cells. The parent flask at PDL 18 was subcultivated at about 2.5×10^6 cells per flask in duplicate for each oxygen concentration. Every 7 d the cells were trypsinised, counted, subdivided and regassed. A solution of 0.25% trypsin in Ca- and Mg-free balanced salt solution (Pacific Biologicals) was used to detach cells from flasks for subcultivation. Cell counts were determined using a haemocytometer, or by Coulter Counter Model P64. When necessary, the pH of the culture medium was adjusted by adding 7.5% sodium bicarbonate. PDL was determined by the split ratio used in the subcultivation and is derived from the equation $n = n_0 2^x$ where n = final cell number in a single culture, n_0 = starting cell number, and x = number of doublings². Once each week, cultures were split at ratios of 1:4 for 2×10^6 or more cells, or 1:2 for 1×10^6 to 2×10^6 cells, and the PDL was estimated by adding 2 population doublings at each 1:4 split or 1 PDL at each 1:2 split. If the number of cells was less than 1×10^6 they were resuspended and reseeded. If cells had not reached confluence, they were fed. When cultures would no longer grow into a confluent monolayer of cells within 3 weeks after a 1:2 split following addition of fresh medium each week, the cultures were considered to be at the end of their lifespan. Δ , 10% O_2 ; $\bullet$, 20% O_2 ; $\circ$, 50% O_2 .

Table 1 Effect of oxygen concentration on the lifespan of human diploid cells

	Population doublings*		Increase (%)
	20% O_2	10% O_2	
WI-38			
Series 32	47	65	38
Series 46A	57.5	67.5	17
Series 46B	56.5	67.5	19
Series 46B	57.5	62.5†	9
IMR-90			
Series 5	39	47	21
Series 6	38	50	32

*Started at PDL 20 or 22; values derived from averaging duplicate cell counts.

†Transferred from 20% O_2 to 10% O_2 at PDL 52.5.

WI-38 and two series with IMR-90 with 10% oxygen, showing that cell lifespan is enhanced on average by about 25%. In one case, series 46B for WI-38 where the oxygen concentration was lowered to 10% only after PDL 52.5, the benefit in lifespan extension was only 9%. This suggests that extension of lifespan by lower oxygen may be realised at any stage in the lifespan, and the effect of oxygen concentration on long term growth may be cumulative.

In other studies not reported here (J. Walton collaboration), we have found that cells maintained under 50% oxygen show extensive morphological damage, particularly to mitochondrial and membranous structures. No apparent morphological changes were observed between ambient oxygen and 10% oxygen, except that the morphological changes usually associated with phase III (the senescence phase), were delayed in cells grown at lower oxygen.

The ambient oxygen concentration of 20% normally used in cell cultures is probably higher than that to which cells are exposed *in vivo*. Although cells have evolved several mechanisms for counteracting oxygen toxicity (namely superoxide dismutase, glutathione peroxidase), the possibility remains that cumulative damage arising from O_2 and products of oxidative metabolism may limit the lifespan of cells both *in vivo* and *in vitro*. Previous studies^{4,5} have demonstrated that the growth of cells after low cell density seeding can be enhanced by lowering the oxygen concentration below ambient; in static culture, clonal growth of WI-38 and adult monkey cells was observed by Taylor *et al.*⁴ to be enhanced by lowering the oxygen in the gas phase from ambient to 1%, and that variable growth and reduced plating efficiencies occurred in cultures grown at ambient 20% oxygen. Similar findings were reported by Richter *et al.*³ both for neoplastic and non-neoplastic cells from foetal tissues. Our results are consistent with these findings. Furthermore, we have now evaluated the long term influence of lower oxygen concentration on growth of normal human diploid cells in static cultures, and show that an average extension of the lifespan of 25% can be achieved by lowering the oxygen concentration from 20% to 10%.

Oxygen is essential, yet it may be inhibitory for growth if its steady-state concentration is below or above optimal. For two normal human diploid cell strains, 10% O_2 seems to provide optimum conditions for long term growth in culture. In a similar study, Balin *et al.*⁶ reported no extension of WI-38 lifespan by O_2 concentrations between 5.6 and 50 mmHg; the effect of 75 mmHg, equivalent to 10% O_2 , was not investigated; furthermore there was no stimulatory effect with low O_2 , and the same inhibitory effect with high O_2 as we have observed⁷ was found. This suggests that further studies on the effects of oxygen and oxidative damage on cells in culture are important. Other factors which have been reported to extend lifespan of normal human diploid cells (compare refs 3,6-8) such as

hydrocortisone, serum, additional bovine serum albumin and vitamin E, should be evaluated alone and in combination, under lower oxygen concentrations. The extent to which cells can be cultivated *in vitro* in more optimal environmental conditions can thus be determined. The wide use of normal human diploid cells as model systems for ageing studies and virus production, for example, indicates that immense benefits could result if such cells could be cultured on a long term or indefinite basis *in vitro*.

We thank Ms Joyce Kirkham for assistance with preliminary experiments and Dr Judie M. Walton for discussion and collaboration on the cytotoxic effects of O_2 . We thank Dr Hayflick (Stanford) for starter cultures of WI-38 and the IMR-90 starter cultures were from the Institute for Medical Research, New Jersey. This research was supported by the Energy Research and Development Administration.

LESTER PACKER
KATHY FUEHR

Membrane Bioenergetics Group,
University of California,
Berkeley, California 94720

Received 31 January; accepted 27 March 1977.

- 1 Hayflick, L. *Expl Cell Res.* 37, 614-636 (1965).
- 2 Nolan, J. S. & Packer, L. *Methods in Enzymology XXXII(B)* (eds Fleischer, S., Packer, L. & Estabrook, R. W.) 561-568 (Academic, New York, 1974).
- 3 Packer, L. & Smith, J. R. *Proc. natn. Acad. Sci. U.S.A.* 71, 4763-4767 (1974).
- 4 Taylor, W. G., Richter, A., Evans, V. J. & Sanford, K. K. *Expl Cell Res.* 86, 152-156 (1974).
- 5 Richter, A., Sanford, K. K. & Evans, V. J. *J. natn. Cancer Inst.* 49, 1705-1711 (1972).
- 6 Balin, A. K., Goodman, D. B. P., Rasmussen, H. & Cristofalo, V. J. *J. Cell Physiol.* 89, 235-249 (1976); *J. Cell Biol.* (in the press).
- 7 Packer, L. *Agings, Carcinogenesis and Radiation Biology* (ed. Smith, K. E.) 519-535 (Plenum, New York, 1976).
- 8 Packer, L. & Smith, J. R. *Proc. natn. Acad. Sci. U.S.A.* (in the press).

Mutation and inactivation of mammalian cells by various ionising radiations

THERE is almost no information on the mutagenic effectiveness of the various ionising radiations for man and only limited information about radiations other than X or γ rays for experimental mammals. We have measured inactivation and mutation induction in cultured mammalian cells exposed to various radiations covering a range of linear energy transfer (LET; ref. 1). We show for two cell types that the maximum mutagenic effectiveness of these radiations, relative to X or γ rays, is about twice that for inactivation. Preliminary experiments suggest that specific chromosome aberrations may be associated with mutations induced by radiations of high LET.

LET is a measure of the deposition of energy along the tracks of the charged particles produced in irradiated material. The distribution of energy loss events along a track depends on the type of radiation; events are widely-spaced for radiations of low LET such as X or γ rays, but more closely spaced for radiations of higher LET such as α particles (as from plutonium) and the protons and other secondary ions produced by neutrons in tissue. The effectiveness of a radiation in producing a particular biological response relative to that of X or γ rays is known as its relative biological effectiveness (RBE; see ref. 2 and Table 1 legend). The yield of mutations per unit absorbed dose of radiation in lower organisms and plants is affected by LET (ref. 2). High dose-rate (acute) irradiations of mice have shown that the RBE of fission neutrons for the production of several types of mutation is about 6 in spermatogonia and about 4.5 in maturing oocytes³. RBEs of about 20 for mutation induction have been found after low dose-rate (chronic) irradiation of spermatogonia by fission neutrons³. But neutron irradiations produce tracks of widely differing LETs (for fission neutrons the range is about 10 to over

500 keV μm^{-1}) and the observed RBEs give little indication of the variation of RBE with LET. Compared with whole mammals, cultured mammalian cells offer the advantages of rapidity of assay and ease of irradiation, especially when the radiation is poorly penetrating, and also the opportunity for more detailed analysis of the mechanisms of mutagenesis. Quantitative estimates of mutation induction by low LET radiation can be made for cultured mammalian cells⁴, including freshly-isolated human cells, using a mutation system based on loss of the activity of the purine salvage enzyme hypoxanthine-guanine phosphoribosyl transferase (HGPRT).

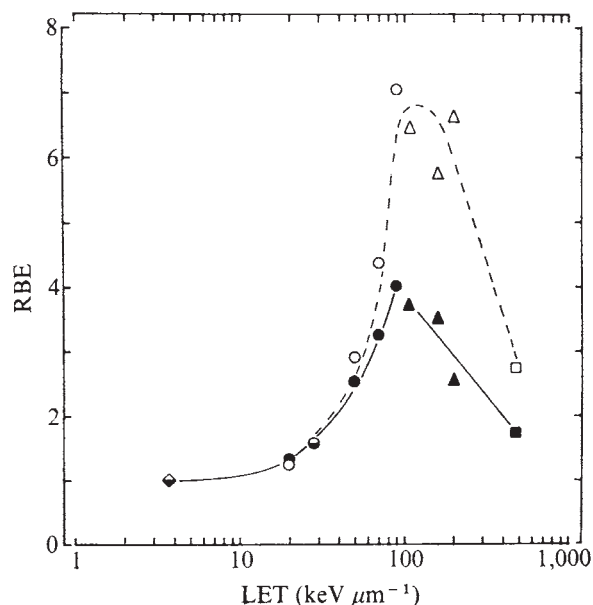
Cultures of freshly-isolated human cells and established V79 hamster cells were irradiated with helium, boron or nitrogen ions covering an LET range of 20-470 keV μm^{-1} . Table 1 gives the coefficients of the dose-response curves for inactivation and mutation by these radiations (see Table 1 legend and ref. 5). The RBE_a is the ratio of the linear terms (α coefficients) of the respective dose-response curves. For hamster cells, however, the RBE varies with dose because the shapes of the dose-response curves change with LET (ref. 5). This is seen in Table 1 which gives values of RBE at low doses (RBE_a) and an example of values at higher doses (RBE_{HD}).

A plot of RBE against LET for the human cells is shown in Fig. 1 and for the hamster cells in Fig. 2. The variation of RBE with LET for inactivation and mutation induction in both cell types shows similar humped forms with maxima in the LET range 85-200 keV μm^{-1} . In this LET range the RBE for mutation induction was about twice that for inactivation. In hamster cells, this approximately twofold difference in RBE values was not influenced by the response level at which RBE was determined (Table 1).

The forms of RBE-LET relationships in Figs 1 and 2 can be explained if mutation or cellular inactivation require that one charged particle should leave either (1) several energy loss events in a single sensitive cellular component or target⁶⁻⁸ or (2) one energy loss event in each of two targets⁹⁻¹². On hypothesis (2) the two targets could be the two strands of a DNA duplex (diameter ~ 2 nm)¹⁰⁻¹².

If the dimensions of the target are small, then differences in the track structure of different ions of similar mean LET,

Fig. 1 RBE-LET relationships for freshly-isolated human diploid fibroblasts. Open symbols, mutation induction; closed symbols, inactivation. $\diamond$, $\blacklozenge$, 250-kVp X rays; $\circ$, $\bullet$, ^4He ions; $\triangle$, $\blacktriangle$, ^{10}B ions; $\square$, $\blacksquare$, ^{14}N ions. Data points are RBE_a values of Table 1. An arbitrary LET value has been assigned to X rays within the range given in ref. 1.



such as track width and LET spectrum, may influence the relative effectiveness of the radiation. Some of our data are consistent with this idea, as seen in the difference between the RBE for boron ions of $110 \text{ keV } \mu\text{m}^{-1}$ (track width $\sim 1,000 \text{ nm}$) and for helium ions of $90 \text{ keV } \mu\text{m}^{-1}$ (track width $\sim 40 \text{ nm}$) for the inactivation of hamster cells (Fig. 2). But, since these differences were not found for the human cells other mechanisms may be involved; for example, differences in the abilities of the two cell types to repair radiation damage^{13,14}.

There is some evidence from the work of others that the

different types of mutation induced by ionising radiation may have different RBE-LET responses. With high LET helium and carbon ions the maximum RBE for deletion mutations in *Neurospora* was greater than that for point mutations². Similarly, in the *d-se* region of the mouse genome, more than twice as many multifunctional mutations were produced by fission neutrons than by X or γ rays¹⁵. These multifunctional mutations are believed to arise mainly as a result of deficiencies (deletions)¹⁵, suggesting a higher neutron RBE for deficiencies than for mutations at single loci.

Table 1 Mutation and inactivation of human and hamster cells by ionising radiations

a Inactivation Radiation type and LET	Human cells		Hamster cells		RBE _α	RBE _{HD}
	$\alpha \times 10^3$ (rad ⁻¹) Best fit (s.e.)	RBE _α	$\alpha \times 10^3$ (rad ⁻¹) Best fit (s.e.)	$\beta \times 10^8$ (rad ⁻²) Best fit (s.e.)		
X rays/ γ rays	7.92 (0.16)	1.0	1.43 (0.15)	2.6 (0.2)	1.0	1.0
Helium ions (LET in keV μm^{-1})						
20	10.9 (0.2)	1.4	4.9 (0.3)	1.8 (1.2)	3.4	1.7
28	12.5 (0.5)	1.6	5.5 (1.1)	4.2 (3.1)	3.8	2.1
50	19.8 (0.3)	2.5	9.3 (1.0)	2.5 (5.4)	6.5	3.0
70	25.7 (0.5)	3.2	9.6 (1.4)	9.7 (7.9)	6.7	3.6
90	31.6 (0.7)	4.0	12.9 (0.8)	7.0 (5.1)	9.0	4.3
Boron ions (LET in keV μm^{-1})						
110	29.6 (1.0)	3.7	7.4 (0.9)	14 (5)	5.2	3.2
160	27.9 (1.4)	3.5				
200	20.0 (0.3)	2.5	11.2 (2.1)	19 (20)	7.9	4.4
Nitrogen ions (LET in keV μm^{-1})						
470	13.8 0.3	1.7	8.8 (0.4)	-1.9 (0.9)	6.2	2.5
b Mutation						
Radiation type and LET	Human cells		Hamster cells		RBE _α	RBE _{HD}
	$\alpha \times 10^7$ (rad ⁻¹) Best fit (s.e.)	RBE _α	$\alpha \times 10^7$ (rad ⁻¹) Best fit (s.e.)	$\beta \times 10^{10}$ (rad ⁻²) Best fit (s.e.)		
X rays/ γ rays	3.10 (0.08)	1.0	0.35 (0.05)	0.86 (0.15)	1.0	1.0
Helium ions (LET in keV μm^{-1})						
20	3.94 (0.08)	1.3	1.0 (0.7)	3.6 (4.7)	2.9	2.3
28	4.85 (0.39)	1.6				
50	8.99 (0.33)	2.9	4.9 (0.6)	8.0 (5.2)	14	6.2
70	13.5 (0.6)	4.3				
90	22.0 (1.7)	7.1	6.0 (2.2)	41 (31)	17	9.5
Boron ions (LET in keV μm^{-1})						
110	20.1 (1.4)	6.5	6.2 (2.7)	30 (27)	18	8.9
160	17.8 (1.2)	5.7				
200	20.7 (0.9)	6.7	6.2 (4.3)	33 (59)	18	9.1
Nitrogen ions (LET in keV μm^{-1})						
470	8.5 0.3	2.8	5.4 (0.5)	-2.4 (2.0)	15	5.3

Cultures of freshly-isolated human fibroblasts (HF19) and an established line of Chinese hamster cells (V79-4) were maintained in Eagles minimal essential medium plus 10% foetal calf serum (Gibco-Biocult) using methods previously described^{20,21}. In most experiments cells were spread on to dishes with thin plastic bases (ICI Melinex), incubated overnight, and then irradiated without medium present. Techniques used for the various radiations are described in ref 5. Track intersection irradiations with ^4He , ^{10}B and ^{14}N ions were carried out at the variable energy cyclotron and the van der Graaf tandem accelerator of the AERE, Harwell. Methods of irradiation and dosimetry were similar to those of Neary²² except that the dishes were held in a wheel which rotated continuously during the irradiation to bring each dish in line with the beam a number of times. The average absorbed dose rate during each irradiation was 40–120 rad min⁻¹ which correspond to instantaneous absorbed dose rates of 2–9 krad min⁻¹ during the time that each dish was in the beam. The LET values are based on stopping power data for water assuming a density of 1 g cm⁻³.

The survival of irradiated cells was usually estimated by their ability to grow and form colonies on the Melinex dishes after incubation periods of 7 d (hamster cells) or 12 d (human cells). Some survival estimations and all mutation estimations were made with cells respread after irradiation on to polystyrene dishes or bulk culture vessels²³ with normal growth medium. Mutants deficient in HGPRT activity were selected by resistance to the cytotoxic purine analogue 6-thioguanine⁴. To detect the maximum number of these mutants amongst irradiated cells, the cells were allowed different periods of post-irradiation growth in normal medium and then were respread into medium containing 6-thioguanine. Factors affecting the detection of radiation-induced mutants of this type have been described in detail elsewhere^{13,14,21,24}. For human cells the logarithm of surviving fraction and the induced mutation frequency per viable cell were both linearly related to dose, while for hamster cells these quantities were most simply described by a quadratic equation. Consequently the data points, weighted by the inverse of their variances, were fitted by the method of least squares to the following equations:

$$\text{hamster cell surviving fraction, } S/S_0 = \exp -(\alpha D + \beta D^2)$$

$$\text{human cell surviving fraction, } S/S_0 = \exp -(\alpha D)$$

$$\text{hamster cell induced mutation frequency, } M = \alpha D + \beta D^2$$

$$\text{human cell induced mutation frequency, } M = \alpha D$$

where D is the dose. The best fits for the coefficients α and β are listed above; the errors shown are simple standard errors (s.e.) of each coefficient in turn with the other at its best fit value. RBE values are derived either as the ratio of α for any given radiation to α for X or γ rays (RBE_α) or, for the hamster cells only, also as the ratio of doses required to produce 10% survival or an induced mutation frequency of 6.8×10^{-5} (this frequency is found after a dose of γ rays yielding 10% survival) (RBE_{HD}). The results given for helium ions of 20 and 28 keV μm^{-1} and for nitrogen ions of 470 keV μm^{-1} are from single experiments; two or more experiments were carried out for the remaining LETs.

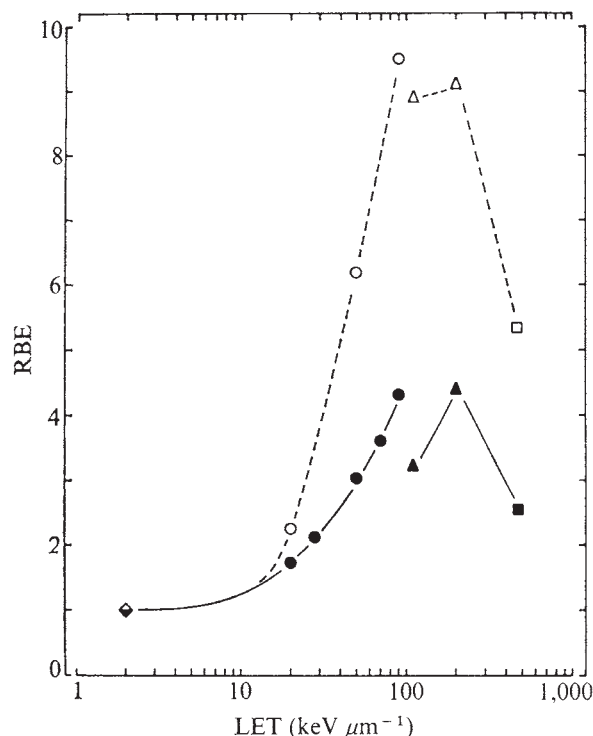


Fig. 2 RBE-LET relationships for established V79 Chinese hamster cells. Symbols as for Fig. 1 except $\diamond$, $\blacklozenge$, ^{60}Co γ rays. Data points are RBE_{HD} values of Table 1. An arbitrary LET value, comparable to that given for X rays in Fig. 1, has been assigned to γ rays within the range given in ref. 1.

No current genetic techniques for somatic cells can distinguish small deletions from point mutations. In man, however, the HGPRT gene is known to be located on the long arm of the X chromosome¹⁶ and gross genetic changes associated with loss of HGPRT may be detectable in mutants of cultured cells by chromosome banding techniques. Our preliminary results with ASG banding¹⁷ show that visible aberrations in the long arm of the X chromosome occur in about 40% of HGPRT-deficient mutants induced by nitrogen ions of high LET. In contrast, X-chromosome aberrations have not yet been detected in spontaneous and X-ray induced thioguanine-resistant mutants.

These preliminary observations suggest that an additional mutagenic mechanism becomes important with high LET irradiations. We previously found a simple relationship between inactivation and mutation induction by low LET radiation in cultured mammalian cells^{4,18}, and an additional mutagenic mechanism operating at higher LETs could explain the higher maximum RBE for mutation induction relative to inactivation (Figs 1 and 2). It is not yet possible to distinguish between two alternative molecular processes which might be involved in this additional mechanism: (1) that a different type of mutagenic lesion is induced by high LET radiation or (2) that lesions similar to those induced by low LET radiation are modified or interact after high LET radiation to give an increased probability of the lesions becoming mutagenic.

The implications for radiobiological theory of the data summarised here will be discussed more fully in subsequent publications. It is clear, however, that these data provide useful empirical confirmation of current radiological protection standards. The recommendations of the International Commission on Radiological Protection on maximum permissible doses for radiation effects in man include 'quality factors' (Q s) which are intended to allow for the effects of LET. The maximum Q is given as 20 (ref. 19), in agreement with the RBE for mutation induction by chronic irradiation in the mouse, and this is sup-

ported by the RBE_{gs} obtained here for mutation of cultured hamster cells by low doses. The cultured human cells showed a maximum RBE for mutation induction somewhat less than this maximum Q . We should stress that the RBE values found for mutation to HGPRT-deficiency in cultured cells are probably averages of the RBEs for more than one type of genetic change, as discussed above. Consequently other mutations, perhaps comprising different combinations of genetic changes, might have RBEs different from those found here.

We thank our colleagues for experimental assistance and for comments on the manuscript, and the staffs of the AERE accelerators, Harwell, for their cooperation.

Note added in proof: We now have evidence that X-chromosome aberrations are associated with thioguanine resistance in a proportion of mutants induced by both low and high LET radiations, but that different types of aberration predominate at different LETs.

ROGER COX
JOHN THACKER
D. T. GOODHEAD
R. J. MUNSON

MRC Radiobiology Unit, Harwell,
Didcot, Oxfordshire, UK

Received 15 March; accepted 5 April 1977.

- 1 International Commission on Radiation Units and Measurements, Rep. 16 (1970).
- 2 International Commission on Radiological Protection, Publ. 18 (Pergamon, Oxford 1972).
- 3 Searle, A. G. *Adv. Radiat. Biol.* 4, 131-207 (1974).
- 4 Thacker, J. & Cox, R. *Nature* 258, 429-431 (1975).
- 5 Cox, R., Thacker, J. & Goodhead, D. T. *Int. J. Radiat. Biol.* (in the press).
- 6 Howard-Flanders, P. *Adv. biol. med. Phys.* 6, 553-603 (1958).
- 7 Brasted, T. *Adv. biol. med. Phys.* 8, 161-224 (1962).
- 8 Barendsen, G. W. *Curr. Top. Radiat. Res.* 4, 293-356 (1968).
- 9 Neary, G. J. *Int. J. Radiat. Biol.* 9, 477-502 (1965).
- 10 Munson, R. J., Neary, G. J., Bridges, B. A. & Preston, R. J. *Int. J. Radiat. Biol.* 13, 205-224 (1967).
- 11 Munson, R. J. & Bridges, B. A. *Int. J. Radiat. Biol.* 24, 257-273 (1973).
- 12 Leenhouts, H. P. & Chadwick, K. H. 4th Symp. Microdosimetry, Verbania Pallanza, Italy, 1973 EUR 5122 (1974).
- 13 Thacker, J., Stretch, A. & Stephens, M. A. *Mutat. Res.* 42, 313-326 (1977).
- 14 Cox, R. & Masson, W. K. *Mutat. Res.* 37, 125-136 (1976).
- 15 Russell, L. B. *Mutat. Res.* 11, 107-123 (1971).
- 16 Brown, J. A. *et al. Cytogenet. Cell Genet.* 16, 54-59 (1976).
- 17 Sumner, A. T., Evans, H. J. & Buckland, R. A. *Nature new Biol.* 232, 31-32 (1971).
- 18 Munson, R. J. & Goodhead, D. T. *Mutat. Res.* 42, 145-160 (1977).
- 19 Recommendations of the International Commission on Radiological Protection, Publ. 15, (Pergamon, Oxford, 1970).
- 20 Cox, R., Masson, W. K. & Bance, D. A. *Lab. Practice* 22, 733-734 (1973).
- 21 Thacker, J., Stephens, M. A. & Stretch, A. *Mutat. Res.* 35, 465-478 (1976).
- 22 Neary, G. J. in *The Uses of Cyclotrons in Chemistry, Metallurgy and Biology* (ed. Amphlett, C. B.) 194-203 (Butterworth, London, 1970).
- 23 Cox, R., Masson, W. K. & Bance, D. A. *Mutat. Res.* 35, 173-178 (1976).
- 24 Cox, R. & Masson, W. K. *Mutat. Res.* 36, 93-104 (1976).

Group and mass effects in diprionid sawflies

THE larvae of gregarious insects species, when reared as single individuals, have a significantly higher death rate than do solitary forms¹. This has been demonstrated in diprionid sawflies, where all main genera have solitary and very gregarious species². It has been argued that this high mortality is due to starvation, because only one in three of the newly hatched larvae of the gregarious *Neodiprion pratti* could feed successfully on the tough pine needles, whereas larvae in a colony could join and enlarge feeding sites, thus benefiting from an elementary 'division of labour'³. Our experiments, however, have not confirmed such a mechanistic explanation.

Improved rearing techniques kept larval mortality at ~10% for most species, including the solitary *Diprion frutetorum* and the scarcely gregarious *N. abietis*. A consistently high mortality was encountered only in individually reared first instars of *N. lecontei*, which is undoubtedly the most gregarious species we have studied. The most unexpected results were the very low mortality of isolated first instars of the two gregarious species *N. rugifrons* and *N. pratti* (Fig. 1). Although most deaths in these experiments occurred in the first instar, they were not, as has been claimed, caused by starvation. More than 70% of the larvae had fed before they succumbed. Paradoxically, it

was the doomed isolated first instars of gregarious forms that produced many more feeding incisions than did the larvae that were reared in groups (Fig. 2). Psychological factors entered into the situation to change the behaviour of the larvae. These were indicated by an increased restlessness of the larvae which wandered for long periods between each feeding bout and sometimes left the foliage altogether. This disjointed behaviour could be stabilised by the addition of more larvae to the experimental situation, until a species-specific group size was attained when feeding became normal and mortality was low. This threshold was reached with five larvae in *N. lecontei* on red pine, but with a smaller group size on jack pine, the optimal host available to this group (Fig. 1).

Colonial feeding in social animals is often associated with better weight gain and thus shorter rates of development. No such effect was noticed in *N. lecontei* when isolated and group-reared animals were compared. Singly reared *N. pratti* larvae took 30% more time to complete their feeding stages than their brother and sister reared in aggregations. The ecological implications of a mechanism that reduced the exposure time to enemies are obvious, although by no means the only benefits accruing from a gregarious habit. The larvae discourage their attackers by the discharge of a viscous, repellent liquid and the synchronous beating of head and thorax. These activities not only result in reduced losses but also in a disproportionately high survival of individuals in the centre of the colony, at the expense of those located at the periphery⁴.

The intensity of aggregative behaviour in diprionid larvae is eclipsed by other sawflies, many of them confined to the Southern Hemisphere. Females of the Brazilian *Themos olfersii* (Argidae)⁵ and the Australian *Pseudoperge guerini*⁶ guard not only their eggs but also their young larvae for several weeks. Cooperation in the *Pseudoperge* larvae is especially important just after hatching when they have to fight their way through the tough epidermis of the *Eucalyptus* leaves in which the egg pods are placed. Similarly, mature larvae have to penetrate the hard crust of the soil to spin their massed cocoons underground. Larger groups clearly succeed much better at both tasks. Cohesiveness and conspecific attraction of *Pseudoperge*

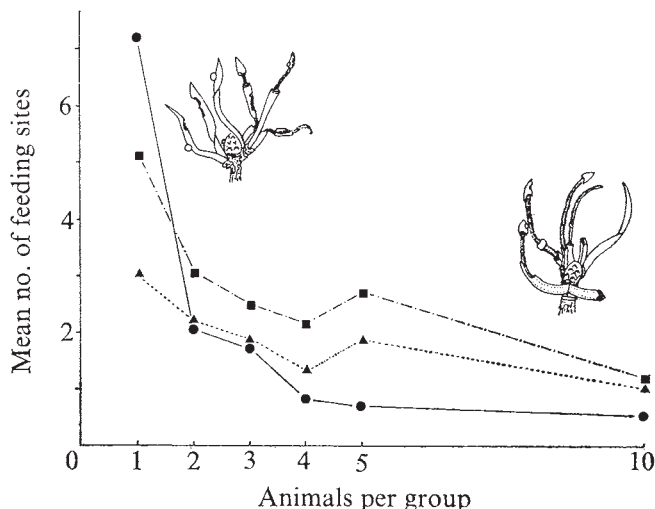


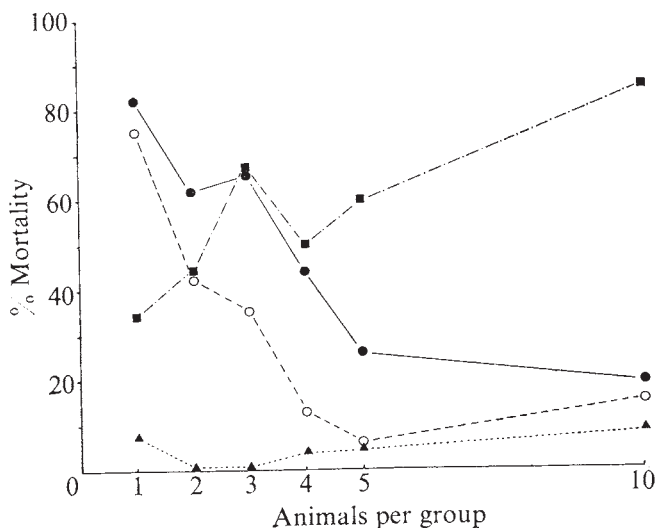
Fig. 2 Feeding patterns of *Neodiprion* larvae 1–6 d old. The mean numbers of feeding sites per larva were significantly different for each species ($P < 0.001$). Large feeding aggregations resulted in normal feeding patterns. In small groups the gregarious *N. lecontei* showed disjointed behaviour resulting in scattered feeding sites (compare the two drawings) accompanied by high mortality (Fig. 1). The difference in number of feeding sites is significant ($P < 0.001$) between groups of three and four larvae for *N. lecontei*, whereas for *N. abietis*, the scarcely gregarious species, such a difference exists only between one larva and a group of two. These differences correspond to species-specific group sizes. No such effects were observed for *N. pratti*, thereby explaining its low mortality. ●, *N. lecontei*; ▲, *N. pratti*; ■, *N. abietis*.

larvae is unrivalled by Northern Hemisphere sawflies. Fifty larvae of all ages spaced regularly on a paper grid 50 cm × 50 cm coalesced within 25 min to form an intertwining mass inseparable without the use of anaesthesia. Gregarious *Neodiprion* larvae by contrast, showed only feeble interest in one another when similarly tested. The presence of food caused the formation of large, stable groups but even dry, inedible foliage encouraged temporary groups of two or three larvae to be formed.

Although gregarious behaviour, or its absence, has led to obvious morphological and behavioural modifications, it seems that the greatest impact for most species concerns their ecology and population dynamics. The tenthredinid sawfly genus *Pikonema* illustrates this hypothesis well. Two related species feed on spruce, but whereas *P. alaskensis* is common and kills trees, *P. dimmockii* is never abundant or a threat to its host and the reason for this is found in the behavioural patterns of the larvae. The gregarious *P. alaskensis*, unlike the solitary *P. dimmockii*, does not get alarmed by frequent contacts with other larvae but resumes feeding soon after contact. *P. dimmockii* larvae wander for long periods after each encounter and settle down only with difficulty⁷. This suggests that two opposite types of density-dependent adaptations have evolved in larval sawflies. The reciprocal sensory stimulation in the larvae of gregarious species causes a group effect⁸ that enhances the optimal development. A similar stimulation creates a mass effect⁹ with often lethal consequences in solitary species. Similar subtle pressures seem to have operated in the rearing experiments with *N. abietis* (and *D. frutetorum*), where a clear relationship was established between increasing population density and higher larval mortality (Fig. 1). The primitive sociality of some gregarious species is of special interest since interactions between group mates of a feeding aggregation may eventually explain the emergence of cooperation in the complex societies of wasps, bees and ants.

This work was supported by the NRC, Ottawa. We thank Dr J. Fox, Department of Sociology, York University for

Fig. 1 Larval mortality of *Neodiprion* spp. in six experimental situations. A group effect was observed in *N. lecontei*, where an increase in the feeding aggregations resulted in a linear decrease in mortality up to a species-specific group size (which was five larvae on red pine, the natural host, but only four on jack pine). *N. abietis* showed a mass effect, whereas mortality in *N. pratti* was unrelated to group size. ●, *Neodiprion lecontei* on red pine (*Pinus resinosa*); ○, *N. lecontei* on jack pine (*P. banksiana*); ▲, *N. pratti* on jack pine; ■, *N. abietis* on balsam fir (*Abies balsamea*).



the statistical analysis and M. Ondrak and P. Schefter for technical assistance.

MARGARETE KALIN
GERD KNERER

Department of Zoology,
University of Toronto,
Toronto, Canada

Received 14 January; accepted 12 April 1977.

- ¹ Wilson, E. O. *Sociobiology*, (Harvard University Press, Cambridge, Massachusetts, 1975); *The Insect Societies*, (Harvard University Press, Cambridge, Massachusetts, 1971).
- ² Knerer, G., & Atwood, C. E. *Science* 179, 1090–1099 (1973).
- ³ Ghent, A. W. *Behaviour* 16, 110–148 (1966).
- ⁴ Tostowaryk, W. *Canad. Ent.* 104, 61–69 (1972).
- ⁵ de Souza Dias, B. F. *Studia Ent.* 18, 401–432 (1975).
- ⁶ Carne, P. B. *Proc. ecol. Soc. Austral.* 1, 75–78 (1966).
- ⁷ Atwood, C. E. *Symp. Genet. Biol. Italica*, 10 250–260 (1962).
- ⁸ Grassé, P. P. *Experientia* 2, 77–82 (1946).
- ⁹ Schwerdtfeger, F. *Autökologie* (Paul Parey, Hamburg and Berlin, 1963).

Oestradiol and its biosynthesis in *Phaseolus vulgaris* L.

UNTIL recently the presence of steroidal oestrogens in plants has been a matter for debate¹, and any physiological significance associated with them has been generally disregarded^{2,3}. Although steroidal oestrogens have been found in extracts of a few species⁴, the identification of oestrone in *Phaseolus* was based on equivocal procedures⁵. Moreover, as with any compound present in low concentration, it is possible that at least some of the oestrogens identified in plant extracts are attributable to external contamination. This risk can be eliminated by demonstrating the incorporation of radioactive label from a known precursor into the compounds of interest. We report here the incorporation of radioactive isotopes from (3RS)-2-¹⁴C-mevalonic acid DBED (MVA), 4-¹⁴C-oestrone and potassium 6,7-³H-oestrone sulphate into oestradiol by *Phaseolus vulgaris* L., cultivar Canadian Wonder. This represents the first case known to us of successful labelling of an identified steroidal oestrogen from ¹⁴C-MVA in a higher plant. It seems therefore that this class of mammalian sex hormone is produced in plants as well as animals.

Eight-day-old seedlings of *P. vulgaris* were sectioned under water at the base of the hypocotyl, stood in aqueous solutions of the various radioactive substances and allowed to transpire for 5 d (ref. 6). They were then planted in potting compost and grown for 2–5 weeks to facilitate normal growth and development. Harvested plants were solvent extracted (chloroform : methanol : : 2 : 1) under reflux in a Soxhlet apparatus for 8 h. The extract was reduced to dryness *in vacuo* and taken up in ether. The phenolic fraction was isolated by partitioning with 1N NaOH, followed by acidification of the aqueous phase and repartitioning into ether. Thin-layer chromatography (TLC) and column chromatography on Sephadex LH-20 were used to isolate those fractions expected to contain oestrone (E₁) and oestradiol (E₂).

The chromatographic mobilities of metabolites were compared with authentic compounds using TLC on three solvent systems (chloroform : methanol : : 95 : 5; benzene : propan-2-ol : : 95 : 5; petroleum ether : ethyl acetate : : 75 : 25), column chromatography on LH-20 of the underivatised extracts and radio-gas-liquid chromatography (radio GLC) on OV-101 of the trimethylsilyl ether derivatives. Samples believed to contain oestradiol were diluted with non-radioactive carrier oestradiol and recrystallised to constant specific activity.

Using these techniques in combination, ¹⁴C was observed to be incorporated into oestradiol from MVA and from 4-¹⁴C-oestrone. Similarly, oestradiol could be labelled from the

³H-oestrone sulphate. Attempts to label oestrone have not been conclusive; the rate of incorporation from radio-active MVA into the putative oestrone peak was much less than that observed for oestradiol.

One previous report describes the incorporation of ¹⁴C-MVA into a phenolic fraction by the higher plant *Haplopappus heterophyllus*, but the radioactive phenolic material did not correspond to any common steroidal oestrogen⁷. There have been many studies of the biotransformation of the mammalian steroid hormones progesterone and testosterone^{8,9}, but our findings seem to represent the first report of a metabolic transformation of an oestrogen in plants—reduction of the 17-keto steroid E₁ to the 17β-hydroxyl compound E₂.

Evidence of unlabelled oestradiol in extracts of seeds, vegetative and flowering plants was obtained using a modified radioimmunoassay (RIA) technique⁹ and combined gas chromatography-mass spectrometry (GC-MS). The GC-MS analysis¹⁰ involved continuous monitoring at the collector of the ion abundance at m/e 416 (molecular ion of the bis-trimethylsilyl derivative of oestradiol) as generated in the ion source from the GLC effluent of an SE-30 column operated at 250 °C. This process, commonly referred to as single-ion monitoring, enabled us to use the GS-MS system as a semi-specific detector for oestradiol.

This identification contrasts with the results of Kopcewicz⁷ who presented TLC evidence for oestrone in *P. vulgaris* but could not detect oestradiol in the extracts. Indeed, this is only the second report of oestradiol detected as a plant steroid, Awad¹¹ having isolated both oestrone and oestradiol from seeds of *Prunus*.

The verification of the endogenous biosynthesis of a steroid oestrogen in *P. vulgaris* opens the way to further studies of the suggested correlation between the quantitative levels of these compounds in this species and the onset of flowering⁷. Preliminary data obtained by RIA suggest that oestradiol is present at 2–10 μg kg⁻¹ fresh weight in seeds and leaves. It should also be possible to examine the overall pathway of biosynthesis of steroids in plants in order to investigate the presumed homology between plants and animals⁴. In addition, the potential for hormonal heterophylly¹² with respect to oestrogens in plants requires attention.

We thank Dr B. Cook for assistance with RIA and Professor K. Overton and J. Dalziel for radio-GLC facilities. The GC-MS instrumentation (AEI MS30) was provided by grants from the SRC, and I.J.Y. was financed by a post-graduate award from the SRC.

I. J. YOUNG

B. A. KNIGHTS

J. R. HILLMAN

Botany Department,
University of Glasgow,
Glasgow, UK

Received 9 March; accepted 15 April 1977.

- ¹ Jacobsohn, G. M., Frey, M. S. & Hochberg, R. B. *Steroids* 6, 93–99 (1965).
- ² Heslop-Harrison, J. in *Plant Physiology, A Treatise* (ed. Steward, F. C.) 6C, 133–271 (Academic, New York and London, 1972).
- ³ Zeevaart, J. A. D. A. *Rev. Pl. Phys.* 27, 321–348 (1976).
- ⁴ Heftmann, E. *Phytochemistry* 14, 891–901 (1975).
- ⁵ Kopcewicz, J. *Phytochemistry* 10, 1423–1427 (1971).
- ⁶ Atallah, A. M., Aexel, R. T., Ramsay, R. B., Threlkeld, S. T. & Nicholas, H. J. *Phytochemistry* 14, 1927–1932 (1975).
- ⁷ Bennett, R. D., Lieber, E. R. & Heftmann, E. *Pl. Physiol.* 42, 973–976 (1967).
- ⁸ Stohs, S. J. & Rosenberg, H. *Lloydia* 38, 181 (1975).
- ⁹ Dean, P. D. G., Exley, D. & Goodwin, T. W. *Phytochemistry* 10, 2215–2216 (1971).
- ¹⁰ VandenHeuvel, W. J. A., Smith, J. L., Albers-Schönberg, G., Plazonnec, B. & Bélanger, P. in *Modern Methods of Steroid Analysis* (ed. Heftmann, E.) 199–219 (Academic, New York, 1973).
- ¹¹ Awad, O. *Phytochemistry* 13, 678–679 (1974).
- ¹² Burdette, W. J. in *Invertebrate Endocrinology and Hormonal Heterophylly* (ed. Burdette, W. J.) 331–337 (Springer, Berlin, 1974).

Direct evidence of the capacity of the XY germ cell in the mouse to become an oocyte

INDIRECT evidence of the capacity of a Y-containing germ cell to become functional oocyte in ovarian tissue of a chimaeric XX/XY female mouse has been published¹. This animal was one of two fertile XX/XY females obtained by aggregation of early morulae of the inbred strains AKR (albino) and CBA-T6 (agouti). Mated to a BALB/c (albino) male she had given birth to two litters that included one albino son, the remaining six young being agouti. Afterwards she had been retained for observation in connection with another investigation. In consequence she was more than 12 months old when her chromosomes were examined and she was found to contain both XX (CBA) and XY (AKR) cells. Only then was it realised that this implied that the egg that gave the albino son had come from a Y-bearing oocyte. Although ancillary evidence effectively excluded the possibility of erroneous recording or mixture of animals as explanations, there may have been some reluctance to accept the conclusion that a Y-bearing germ cell had become a functional oocyte. It is therefore satisfying to report here the direct observation of an XY oocyte at diakinesis in a preparation from another fertile, chimaeric XX/XY female mouse.

The animal was one of a series of chimaeras obtained by aggregation of embryos from matings chosen with another purpose in view². When breeding tests and other observations had been completed cultures were set up from tail blood of some of the animals and two females that had bred successfully were found to be XX/XY chimaeras. The one (M 9263) had an XY 'pigmented' component with a standard karyotype and an XX 'albino' component marked by a single large metacentric chromosome (Rb1 Ald); the other (M 9290) had an XX 'pigmented' component and an XY 'albino' component homozygous for Rb1 Ald. Both underwent a more detailed chromosome study that included various somatic tissues and also meiosis in oocytes, using preparations made by a modification of Tarkowski's³ method following brief *in vitro* culture⁴. One oocyte from M 9263 at diakinesis (Fig. 1) contained 19 normal bivalents, plus two univalents corresponding closely in relative size to the univalent X and Y chromosomes seen frequently in spermatocytes. Any doubt that these two univalents may have represented a deficient chromosome (not necessarily X) and its normal homologue is removed by the presence of 19 normal bivalents, and the absence of the Rb1 Ald trivalent seen in the remaining oocytes (Fig. 2).

The discovery of this XY oocyte in diakinesis, we hope,

Fig. 1 Oocyte at diakinesis from XY component of M 9263. 19 bivalents plus X and Y univalents (arrowed). Bar represents 10 nm.

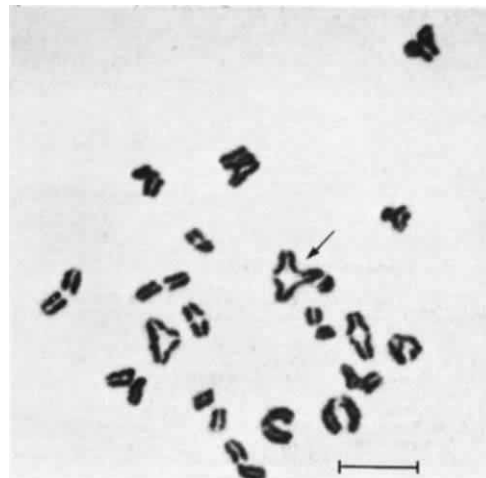
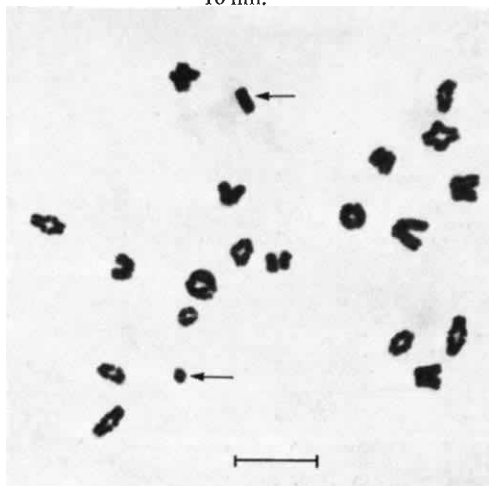


Fig. 2 Oocyte at diakinesis from XX component of M 9263. 18 bivalents plus Rb1 Ald trivalent (arrowed). Bar represents 10 nm.

will remove any scepticism that may remain concerning origin of the exceptional albino son of the earlier report from a Y-bearing oocyte. The cytogenetic evidence showed that the son had received his Y chromosome from his mother and also, surprisingly, that he had the rare 41, XXY ('Klinefelter') karyotype¹. The possibility could therefore not be excluded that a chance non-disjunctional event during oögonial mitosis had given an XXY oocyte that had contributed an X and a Y chromosome to the zygote by secondary non-disjunction.

The alternative was to assume primary non-disjunction in an XY oocyte. Should the first explanation be correct, survival and maturation of the XXY oocyte may have been favoured, even made possible (in view of the presence of the Y chromosome), by the presence of the two X chromosomes, and the event could then have been unique and of trivial significance. The new observation, however, shows that an XY oocyte can at least develop to the point where its germinal vesicle is capable of resuming meiosis during the brief period (4 h) of culture *in vitro*. This normal response to culture and the normal appearance of the chromosomes, moreover, strongly suggest that the oocyte was developing naturally and that it would have been capable of completing normal maturation, including release from its follicle, transport to the oviduct and fertilisation.

The presence of univalent X and Y in the exceptional oocyte (see Fig. 1) gives a hint, and only a hint, that pairing of X and Y in oocytes may be less assured than in spermatocytes, thereby increasing the likelihood of non-disjunction and rendering more plausible the assumption of primary non-disjunction in an XY oocyte.

Relevant data from the four known fertile XX/XY females are summarised in Table 1. The 22 scorable oocyte preparations were obtained from 25 oocytes set up in culture. Breeding records and direct cytological examination together indicate actual or potential functional capacity of 173 XX oocytes and two XY oocytes. This gross disproportion may at first suggest that XY germ cells, though capable of normal function, are nevertheless at a strong disadvantage compared with XX germ cells. Further consideration, however, indicates that such a conclusion would be premature.

The literature provides records of 36 XX/XY chimaeric mice constructed by aggregation of morulae and representing balanced combinations⁵ of component strains: 21 of them were fertile males, six sterile males, three intersexes, four were sterile females, and only two were fertile females⁶⁻¹⁰. Most XX/XY chimaeras evidently develop as males and it is reasonable to suppose that the XX/XY

Table 1 Observations on fertile XX/XY female chimaeras

Mouse number	Age when killed (months)	Sex chromosomes and estimated percentage composition of soma		Offspring		Sex chromosomes of oocytes	
		Pigmented component	Albino component	Pigmented	Albino	XX	XY
9263	13	XY 2	XX 98	0	64	12	1
9290	13	XX 82	XY 18	66	0	9	0
15	15	XX 65	XY 35	6	1	} Not studied	
16	17	XX 75	XY 25	16	0		

The chimaeras were obtained by aggregation of morulae from pigmented and albino stocks. The percentage composition of the soma was estimated as the mean of estimates of proportions of mitotic cells of the two types in direct chromosome preparation from the lymphomyeloid complex, corneas and gut epithelium (mouse 9263); or from lymphomyeloid complex and corneas only (mouse 9290); or the percentage of agouti in the coat (mice 15 and 16)¹. The offspring were derived from matings to albino males. The sex chromosomes of the oocytes were identified in direct chromosome preparations.

females are those that, by chance, contained relatively few XY cells as embryos. It is therefore not surprising to find that the mean proportion of the XY component in the soma, derived from the estimates for the four individual XX/XY females (Table 1), is only 20%. If the proportion of XY cells is assumed to be the same in germ line and soma, the probabilities of drawing a single XY oocyte (or none) in random samples of 77 (chimaera 9263), and seven (chimaera 15), are given by the sums of the first two terms of the binomials $(0.98+0.02)^{77}$ and $(0.65+0.35)^7$, and are 0.54 and 0.23 respectively. Furthermore, breeding tests show that even in XX/XX and XY/XY chimaeras the germ cells are frequently derived from only one of the two components¹¹. In the series from which M 9263 and M 9290 were taken, only eight of the 15 female chimaeras had mixed progenies. Of the remaining seven, one died before tests were completed and four proved on chromosomal examination to be XX/XX, only the two animals described here being XX/XY. The lack of evidence of functional XY ova in two of the four fertile XX/XY females would not therefore be remarkable even if XX and XY oocytes had equal potential for maturation and function.

The rarity of functional XY oocytes in fertile XX/XY female chimaeras cannot therefore yet be taken to indicate that the XY germ cell is at a disadvantage of some kind when it finds itself in a potentially competitive position with XX germ cells either during early proliferation and migration in the developing embryo or later in the ovarian environment. A disadvantage would nevertheless not be surprising in view of the evidence that both X chromosomes are normally active in XX oocytes¹²⁻¹³, and that early embryos derived from XO mothers develop more slowly than those derived from XX mothers¹⁴.

The decisive demonstration that XY germ cells can become functional oocytes means that the developmental fate of the primordial XY germ cell is not irrevocably determined before its migration to the gonads is complete. Evidently the sex of a germ cell is not an autonomous property but is determined by the nature of the gonad in which it finds itself. Our demonstration provides further evidence to counter recent criticism¹⁵ of our original report.

We thank P. H. Glenister for construction of chimaeras.

E. P. EVANS

C. E. FORD

MRC Scientific Staff,
Sir William Dunn School of Pathology,
South Parks Road, Oxford

M. F. LYON

MRC Radiobiology Unit,
Harwell, Didcot, Oxfordshire, UK

Received 11 February; accepted 1 April 1977.

- 1 Ford, C. E. *et al. Proc. R. Soc. B* **190**, 187-197 (1975).
- 2 Lyon, M. F., Glenister, P. H. & Lamoreux, M. L. *Nature* **258**, 620-622 (1975).
- 3 Tarkowski, A. K. *Cytogenetics* **5**, 394-400 (1966).
- 4 Edwards, R. G. *Nature* **208**, 349-351 (1965).
- 5 Mullen, R. J. & Whitten, W. K. *J. exp. Zool.* **178**, 165-176 (1971).
- 6 Myskowska, E. T. & Tarkowski, A. K. *J. Embryol. exp. Morph.* **20**, 33-52 (1968).
- 7 Myskowska, E. T. & Tarkowski, A. K. *J. Embryol. exp. Morph.* **23**, 393-405 (1970).

⁸ Milet, R. G., Mukherjee, B. B. & Whitten, W. K. *Can. J. gen. Cytol.* **14**, 933-941 (1972).

⁹ McLaren, A. J. *Embryol. exp. Morph.* **33**, 205-216 (1975).

¹⁰ Ford, C. E. *et al. Differentiation* **2**, 321-333 (1974).

¹¹ McLaren, A. *Mammalian Chimaeras* 46 (Cambridge University Press, Cambridge, London & New York, 1976).

¹² Epstein, C. J. *Science* **163**, 1078-1079 (1969).

¹³ Gartler, S. M., Liskay, R. M., Campbell, B. K., Sparkes, R. & Grant, N. *Cell Differ.* **1**, 215-218 (1972).

¹⁴ Burgoyne, P. S. & Biggars, J. D. *Dev. Biol.* **51**, 109-117 (1976).

¹⁵ Gordon, J. J. *exp. Zool.* **198**, 367-374 (1976).

The XY chromosome pair in mouse and human spermatocytes, visualised by silver staining

THE nature of the relationship between the X and Y chromosome in human spermatocytes has not been clearly explained by the extensive cytological studies of the last 50 yr (see refs 1-3 for reviews). The sex chromosomes of man and other mammals form a condensed mass (the so-called 'sex vesicle') during zygotene-pachytene in spermatocyte nuclei. Because of its density, the internal structure of the sex vesicle could not previously be studied by light microscopy. We describe here a technique which clearly stains the sex chromosomes within the condensed sex vesicle.

Testicular biopsies of C57BL/6 and BALB/c mice, and of human males were processed by the technique of Evans *et al.*⁴. The material was placed in 1% sodium citrate, teased apart with dissecting scissors to free the tubules, and left for 1 h. After centrifugation, the supernatant was gently pipetted off and the pellet fixed with a fresh mixture of 3:1 methanol:acetic acid for 30 min. The fixative was changed twice. Chromosome preparations were made by the air drying technique.

The silver staining procedure is simplified from a technique of Howell *et al.*⁵, developed to demonstrate nucleolus organiser regions on somatic chromosomes. It consists of two basic steps: (1) Air-dried preparations are treated with three or four drops of 33% aqueous AgNO₃, pH 5.5, under a coverglass for 6-10 min beneath a photoflood light at a temperature of 50-70 °C. After cooling, the AgNO₃ is washed off with deionised water and the preparation is again air dried. (2) Working at room temperature, two drops of ammoniacal silver (8 g AgNO₃ dissolved in 10 ml H₂O, then added to 15 ml concentrated NH₄OH) are placed on the chromosome preparation and immediately followed by two drops of 3% acetate-neutralised formalin. A cover glass is added. The pH of the combined fluids should be 12-13. As soon as the preparation turns a deep yellow (10-60 s) the silver solution is quickly washed off, and the slide made permanent.

After this treatment, the sex vesicle can be seen as a prominent structure, situated at the periphery of the nucleus, and frequently slightly dissociated from it (presumably by the hypotonic treatment).

In the mouse, the two sex chromosomes are seen as thin, positively stained threads, each almost symmetrically curled back on itself, the longer (presumptive X) being readily

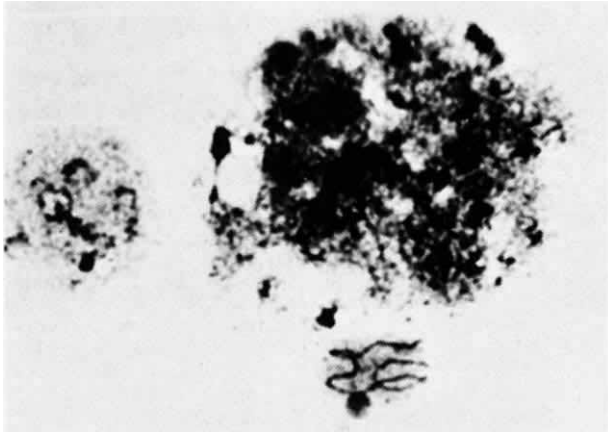


Fig. 1 Pachytene nucleus from a male mouse. X and Y chromosomes are clearly seen within the sex vesicle; also the nucleolus-like body associated with X.

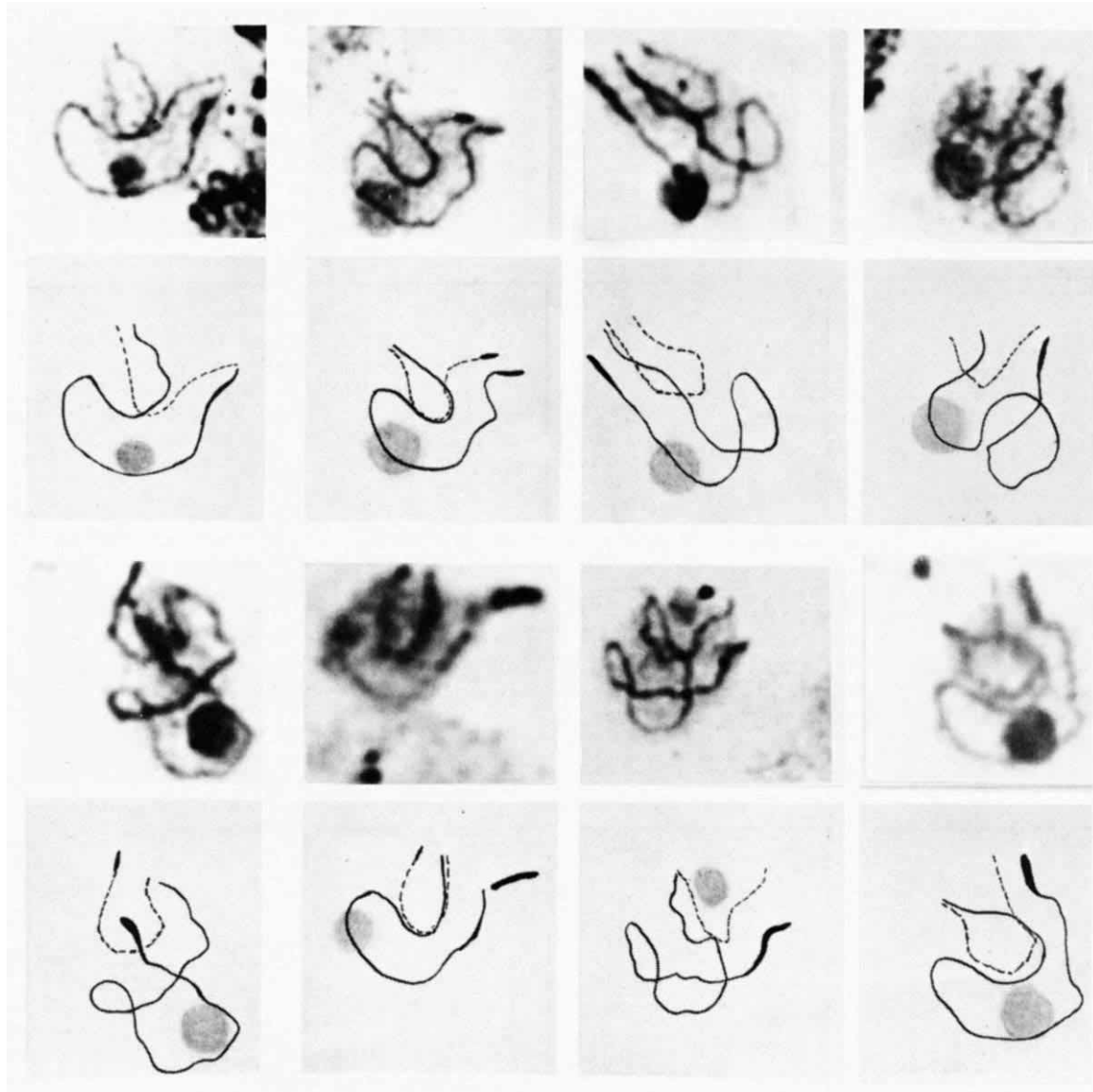
distinguished from the shorter (presumptive Y). Both ends of both chromosomes usually seem to be fixed to the periphery of the sex vesicle not very far from one another. One end of the X, and one end of the Y, are particularly

close to one another, and here the chromosomes seem to run in parallel for a short distance in what may be a parasynaptic association. In addition to this association, the Y in many cells crosses the X again at approximately the same place along its length (see Figs 1 and 2).

At a point about one third along its length the X chromosome is quite regularly associated with a round body which looks like a nucleolus. At pachytene this sphere stains lighter than the surrounding material, although with acridine orange it looks very pale.

In man, the figure has features in common with that of the mouse, although in the early stages of meiotic prophase the sex chromosomes are curiously intertwined and although no nucleolus is evident in the vesicle. The sex bivalent is folded so that the X chromosome forms a rod with the presumptive centromere near the nuclear membrane, and the distal tip is associated with the Y chromosome. Synapsis between the long arms of both chromosomes would then be particularly easy (Fig. 3). In later stages, the bivalent looks much as it does at diakinesis, but more elongated. Generally, a third of the long arm of the X chromosome runs alongside the long arm of the Y chromosome. The X centromere and the distal part of the long arm of the Y are clearly seen, while the other bivalents are still almost indistinguishable.

Fig. 2 Sex bivalents from spermatocytes of the mouse at pachytene and interpretative diagrams. In the diagrams the X chromosomes are shown as continuous, the Y chromosomes as discontinuous.



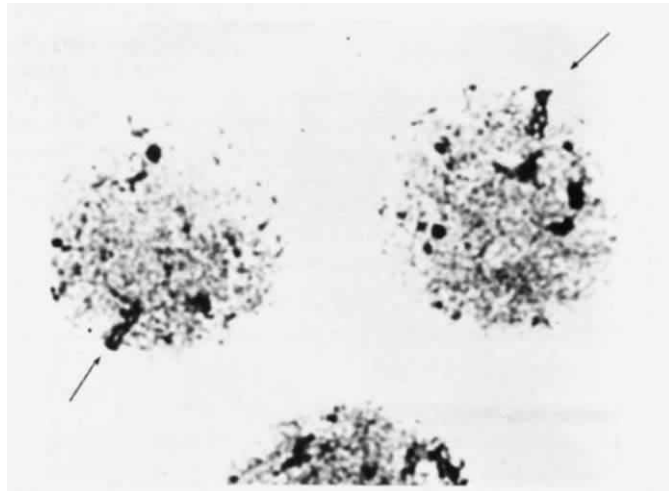


Fig. 3 Sex bivalents (arrowed) in spermatocytes from a normal human male at pachytene.

The sex vesicle is usually densely stained, but in favourable circumstances the curled up sex bivalent has been seen within it⁶. Our observations confirm the side-by-side configuration of segments of the sex chromosomes suggested by previous work. The pictures obtained by this relatively simple light microscope technique are very similar to those previously obtained by three dimensional reconstructions of electron microscope sections³.

The question about the existence of chiasmata between the human X and Y chromosomes has not yet been resolved². After diplotene, the sex pair is seen to be joined in an end-to-end relationship, which may be a terminalised chiasma. A common end region expressed as a short synaptonemal complex is present in the mouse and the human².

Detailed studies of the sex vesicle in pachytene by the method we have described could aid the analysis of structural rearrangements of the sex chromosomes in the mouse and other mammals and might complement clinical diagnosis in males with sex chromosome aberrations or infertility (Fig. 4).

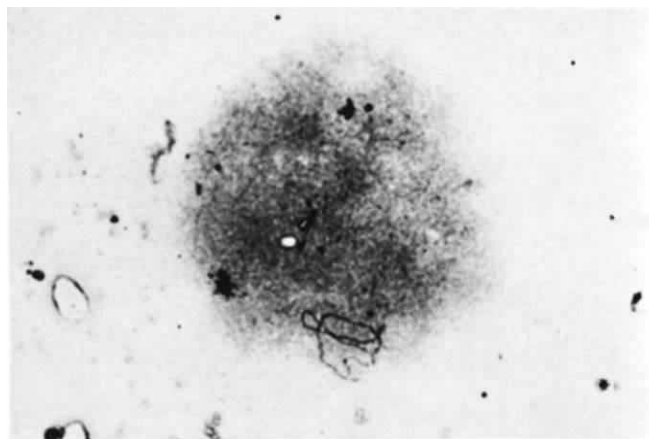


Fig. 4 Sex bivalent in a spermatocyte at pachytene from an azoospermic human male.

This work was supported by the CNRS. We thank Y. Clermont, J. M. Luciani, B. Dutrillaux, Ch. Ford and M. Bobrow for discussions and assistance.

BERNADETTE QUACK
B. NOEL

Laboratoire de Cytogenétique,
Centre Hospitalier,
Chambery 73011, France

Received 22 March; accepted 31 March 1977.

¹ Reitalu, J. *Hereditas* 64, 283-290 (1970).

² Solari, A. J. & Tres, L. J. *Cell Biol.* 45, 43-53 (1970).

³ Luciani, J. M. *Annls Génét.* 13, 101 (1970); *Annls Biol. clin.* 32, 83 (1974).

⁴ Evans, E. P., Breckon, G. & Ford, C. E. *Cytogenetics* 3, 289-294 (1964).

⁵ Howell, W. M., Denton, T. E. & Diamond, J. R. *Experientia* 31, 260-262 (1975).

⁶ Jagiello, G. M. & Polani, P. E. *Guy's Hosp. Rep.* 118, 413-431 (1969).

Simpler technique for serological detection of H-Y antigen on mouse lymphocytes

THE serological detection of the H-Y (male) antigen in the mouse has been demonstrated¹ using the cytotoxicity test against mouse spermatozoa, and that work has been confirmed² by the demonstration of H-Y on male (not female) mouse epidermal cells. There are, however, technical difficulties associated with both assays and these are hard to overcome. We report here a simplified method for the serological detection of H-Y antigen on mouse lymphocytes using *Staphylococcus aureus* (SA) as a marker. We do this in the interests of other investigators who may wish to investigate the H-Y system by a relatively simple method.

SA contains protein A, a polypeptide of molecular weight 42,000 which binds to the Fc of IgG molecules from several species³. It is linked covalently to the peptidoglycan in the cell wall of most strains of SA⁴. Kearney *et al.*⁵ have described a method of detecting membrane-associated antigens on lymphoid cells by antibody coupled to protein A of SA. We have used this procedure to detect H-Y antigen expressed on male lymph node cells.

Mice were purchased from the Jackson Laboratory. Anti-H-Y serum was produced in C57BL/6 (B6) female mice by repeated inoculation with 25×10^6 B6 male spleen cells. Serum was obtained 10 d after the seventh inoculation. Anti-H-Y activity was determined by the cytotoxicity test, with mouse spermatozoa as target cells according to the method of Goldberg *et al.*¹. The Cowan I strain of SA containing 1.7% protein A was obtained from Dr R. C. Williams Jr. It was cultured in Todd-Hewitt broth (Difco) at 37 °C and collected after an 18-h incubation on a shaker. The cells were washed three times in phosphate-buffered saline (PBS; 0.02 M PO₄; 0.15 M NaCl, pH 7.2) and then incubated for 3 h at 37 °C in PBS containing 0.5% formaldehyde. The cells were then washed four times in PBS and placed in a boiling water bath (94 °C in Albuquerque, New Mexico) for 2.5 min at a 10% (v/v) cell concentration. This stock suspension was stored at 4 °C in the presence of 0.1% NaN₃. Before use, cells were washed and resuspended in PBS.

The axillary and inguinal lymph nodes were removed from B6 mice. After mincing into a single cell suspension in Eagle's minimal essential medium (MEM, Gibco), they were washed and resuspended to a concentration of 2×10^7 cells per ml. Anti-H-Y (50 µl) serum was added to 0.25 ml of lymph node cells from male mice and incubated for 30 min at 4 °C. To test for specificity of the H-Y antiserum, lymphocytes from B6 female mice were also treated with anti-H-Y serum. A further control consisting of male lymph node cells treated with normal mouse serum was also included. After washing twice with 5 ml (20 volumes) of MEM containing 10% foetal calf serum (FCS; previously heated to 56 °C for 30 min), the cell pellet was mixed with 0.05 ml of SA suspension and incubated for 15 min at 4 °C. To reduce the background number of unattached SA, the mixture was washed three times in 5 ml of MEM, containing 20% FCS, by centrifugation at 200g for 10 min at 4 °C. After the final wash, the pellet was resuspended in 0.25 ml of MEM. Smears were prepared on microscope slides, air dried, fixed in methanol and stained by the Giemsa method. The slides were observed by light microscopy at $\times 400$ and $\times 1,000$.

A lymphocyte was counted positive if five or more SA were attached. Male lymphocytes were never less than 90%

positive. The average background of positive female lymphocytes, with unabsorbed serum, was 10%, with an upper limit of 20%. With the technique described here, we have made eight tests, in each of which male and female lymphocytes were compared. The slides were coded and read by four individuals. All male cells typed H-Y⁺ and all female cells types H-Y⁻, and all readers agreed on all positive and negative readings.

This work was supported by grants from the NIH.

SEI TOKUDA
TERECITA ARRINGTON
ELLEN H. GOLDBERG
JUDITH RICHEY

Department of Microbiology,
School of Medicine,
University of New Mexico,
Albuquerque, New Mexico 87131

Received 14 February; accepted 7 April 1977.

- 1 Goldberg, E. H., Boyse, E. A., Bennett, D., Scheid, M. & Carswell, E. A. *Nature* 232, 478-480 (1971).
- 2 Scheid, M., Boyse, E. A., Carswell, E. A. & Old, L. J. *J. exp. Med.* 135, 938-955 (1972).
- 3 Kronvall, G., Seal, U. S., Finstad, J. & Williams, R. C. Jr. *J. Immun.* 104, 140-147 (1970).
- 4 Sjoquist, J., Movitz, J., Johansson, I. B. & Hjelm, H. *Eur. J. Biochem.* 30, 190-194 (1972).
- 5 Kearney, R., Chia, E. & Basten, A. J. *Immun.* 114, 1143-1146 (1975).

Tamoxifen is a potent 'pure' anti-oestrogen in chick oviduct

OESTROGEN responsive cells contain a specific proteinaceous macromolecule—one receptor—capable of recognising physiologically active oestrogens as well as a number of synthetic oestrogen agonists and antagonists^{1,2}. In the non-stimulated state, the majority of receptors are located in the cytoplasmic fraction of responsive cells^{3,4}, including the chick oviduct⁵. When oestrogen reaches the target cell, it binds to the cytoplasmic receptor causing its 'activation' and the translocation of the hormone-receptor complex to the nucleus. The interaction of receptor with chromatin is thought to facilitate gene transcription and the synthesis of proteins implicated in the changes characteristic of the oestrogenic response^{6,7}. Receptor is retained in the nucleus for varying periods of time depending on the concentration and nature of the oestrogen. In the rat uterus, the length of time that receptor is retained in the nucleus correlates well with a number of parameters of the hormonal response⁸. The initial translocation of receptor to the nucleus is followed by a period of cytoplasmic receptor replenishment^{3-5,9}. This process is very important as it has been implicated in the ability of cells to respond to subsequent oestrogenic stimulation^{10,11}. Anti-oestrogens prevent oestrogens from expressing their full effects on oestrogen target tissues. Recent studies have indicated that some non-steroidal anti-oestrogens (CI-628, nafoxidine and clomiphene) bind to cytoplasmic oestrogen receptor, induce the translocation of the antagonist-receptor complex to the nucleus, but fail to stimulate subsequent cytoplasmic receptor replenishment¹²⁻¹⁴. Oestrogen antagonism would be primarily due to inhibition of cytoplasmic receptor replenishment¹². Further, such a hypothesis could explain the weak oestrogenic activity of these compounds following a single injection when cytoplasmic receptor is translocated to the nucleus, and their subsequent potent anti-oestrogenic activity after serial injections when the low cytoplasmic receptor level is the limiting factor in receptor translocation¹⁵. We present here results which illustrate that this theory of anti-oestrogenic action does not hold for tamoxifen (ICI 46,474) in the chicken oviduct.

Oestrogen withdrawn (4-6 weeks) chickens^{5,16} were

injected with oestradiol benzoate 1 mg per kg body weight and/or tamoxifen 10 mg per kg. Magnum wet weight, DNA content, and cytoplasmic progesterone receptor concentration (increasing after oestrogen administration^{17,18}) were measured either 24 h after a single injection or 24 h after the last of three daily injections. All experimental procedures have been described previously^{5,18}. The simultaneous administration of oestradiol benzoate (1 mg per kg) and tamoxifen (10 mg per kg) resulted in complete abolition of the oestradiol benzoate induced responses. At the dose tested, tamoxifen alone demonstrated no oestrogenic activity (Fig. 1). Further experiments (manuscript in preparation) showed that even at a dose of 1 mg per kg, tamoxifen was capable of causing a significant ($\geq 50\%$) reduction in oestradiol benzoate induced magnum growth and cytoplasmic progesterone receptor concentration. Moreover, tamoxifen is a potent inhibitor of oestradiol induced ovalbumin synthesis, while having no detectable agonist effect of its own (Binart and Lebeau, unpublished).

Although tamoxifen did not induce any measurable oestrogenic response, it led to appreciable changes in the subcellular distribution of oestrogen receptors with the majority of receptor sites being located in the nucleus 24 h after a single injection (Fig. 2). No change in the total cellular oestrogen receptor level was observed at 24 h, in contrast to the increase after oestradiol benzoate and in agreement with the lack of oestrogenic properties of the anti-oestrogen. When the time-dependence of oestrogen receptor distribution was monitored over a 24 h period following injection of tamoxifen 10 mg per kg, it was apparent that approximately 70% of the cytoplasmic receptor was rapidly translocated to the nucleus during the first hour, and that this pattern of distribution was maintained for the entire period studied (Fig. 2a). In this respect, tamoxifen in the chick seemed to behave in a similar manner to the non-steroidal anti-oestrogens in the rat¹²⁻¹⁴, that is, long-term retention of the nuclear receptor and no (or delayed)

Fig. 1 The effect of oestradiol benzoate (E₂B) 1 mg kg⁻¹ and/or tamoxifen (Tam) 10 mg per kg on magnum wet weight (Wt), DNA content (DNA) and cytoplasmic progesterone receptor concentration (PR) 24 h after the final injection. Groups of oestrogen withdrawn chickens (4-6 animals per group) received either 3 injections at 24 h intervals (open bars) or a single injection (full bars). The data represent the mean $\pm$ SD of three groups of chickens and are presented as a percentage of values in non-injected control animals.

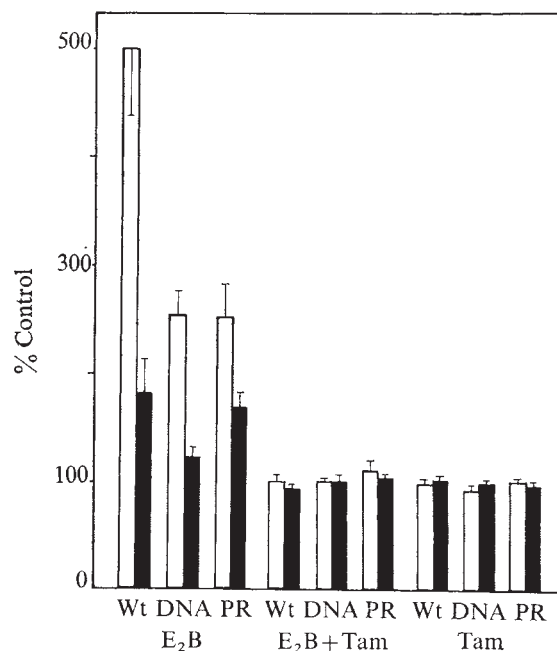
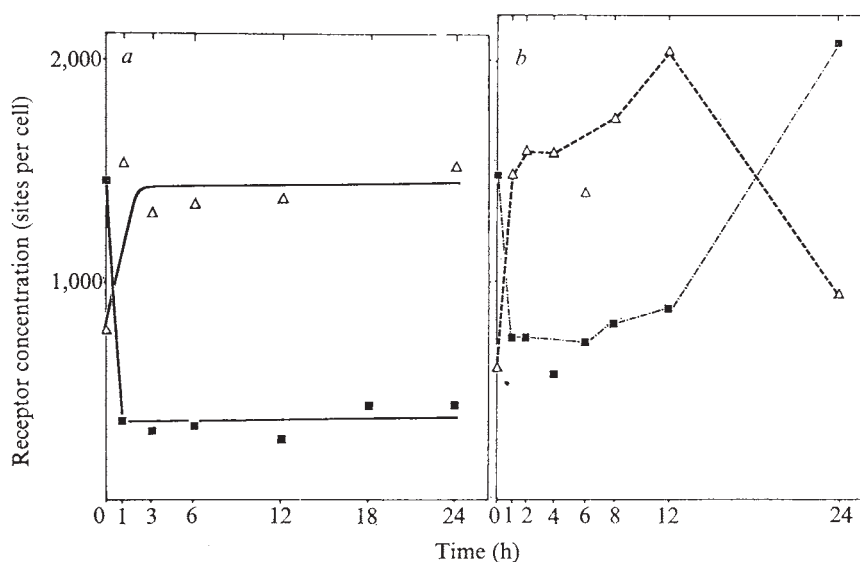


Fig. 2 The effect of a single injection of (a) tamoxifen (10 mg kg^{-1}) or (b) oestradiol benzoate (1 mg kg^{-1}) on the concentration of cytoplasmic (squares) and nuclear (triangles) oestrogen receptor sites during the 24 h following injection.



replenishment of cytoplasmic receptor. In contrast to the rat uterus, however, the presence of oestrogen receptor in the nuclear compartment of chick oviduct cells was not accompanied by any measurable oestrogenic response.

Oestradiol benzoate caused a similar degree of translocation of cytoplasmic receptor to the nucleus during the first hour (Fig. 2b), but nuclear receptor was gradually depleted after 12 h and had fallen to approximately 140% of control values by 24 h. Cytoplasmic receptor levels were replenished and had reached 150% of control by 24 h. At this time, total cellular oestrogen receptor levels had increased to about 160% of their pre-incubation level indicating oestrogen-induced synthesis of new oestrogen receptor. This pattern of receptor subcellular redistribution and synthesis was accompanied by a significant hormonal response (Fig. 1).

These results indicate that a number of currently held theories of the mechanism of action of anti-oestrogenic compounds require re-evaluation. The anti-oestrogenic activity of all non-steroidal anti-oestrogens of the triphenylethylene type cannot be explained simply by their inability to stimulate cytoplasmic receptor replenishment as has been suggested previously¹². Our results show that tamoxifen in the chick oviduct is anti-oestrogenic after a single injection when cytoplasmic receptor levels are not a limiting factor in the amount of receptor translocated to the nuclear compartment. In addition the receptor which was translocated from the cytoplasm to the nucleus was unable to initiate any measurable oestrogenic response. This observation implies that the mere presence of high concentrations of oestrogen receptor in the nuclear compartment is not sufficient for hormone action and suggests, as theoretically more satisfactory, that the ligand plays an important role in determining the final activity of the ligand-nuclear receptor complex. Moreover, these results add support to the theory that there are different populations of nuclear 'acceptor' sites, some of which are biologically inactive^{1,19}. Our data, however, do not support the hypothesis²⁰ that KCl-resistant nuclear sites²¹ designate the specific 'acceptor' sites involved in the expression of maximal oestrogen-induced growth since we were unable to demonstrate any difference between oestradiol benzoate and tamoxifen treated animals with respect to the proportion of nuclear receptor sites which could be extracted with 0.5 M NaCl (manuscript in preparation).

Finally, since tamoxifen, like nafoxidine and clomiphene, is an anti-oestrogen and a weak oestrogen when tested in rodents^{22,23}, some of the discrepancies between the present data and those reported previously for rat uterine system

may be due to species differences in the mechanism of action of these compounds.

We thank Dr A. L. Walpole of ICI for the tamoxifen (Ici 46, 474). This work was supported by the Délégation Générale à la Recherche Scientifique et Technique, the Ford Foundation, and Roussel-Uclaf. R.L.S. acknowledges support from G. D. Searle (Australia) Pty, CSIRO and WHO. We thank Dianne Sutherland for secretarial assistance.

ROB SUTHERLAND
JÁN MEŠTER
ETIENNE-EMILE BAULIEU

Lab Hormones-U 33 Inserm,
94270 Bicêtre, France

Received 7 February; accepted 25 March 1977.

- Baulieu, E. E. *et al.*, *Vitamins Horm.* 33, 649-736 (1975).
- King, R. J. B. & Mainwaring, W. I. P. (eds) *Steroid-Cell Interactions* (Butterworth, London, 1974).
- Jensen, E. V. *et al. Proc. natn. Acad. Sci. U.S.A.* 59, 632-638 (1968).
- Gorski, J., Toft, D. O., Shyamala, G., Smith, D. & Notides, A. *Rec. Progr. Hormone Res.* 24, 45-80 (1968).
- Sutherland, R. L. & Baulieu, E. E. *Eur. J. Biochem.* 70, 531-541 (1976).
- O'Malley, B. W. & Means, A. R. *Science* 183, 610-620 (1974).
- Schimke, R. T., McKnight, G. S., Shapiro, D. J., Sullivan, D. & Palacios, R. *Rec. Progr. Hormone Res.* 31, 175-211 (1975).
- Clark, J. H., Anderson, J. N. & Peck, E. J., Jr, in *Receptors for Reproductive Hormones* 36 (eds O'Malley, B. W. & Means, A. R.) 15-59 (Plenum, New York, 1973).
- Mester, J. & Baulieu, E. E. *Biochem. J.* 146, 617-623 (1975).
- Anderson, J. N., Peck, E. J., Jr, & Clark, J. H. *Endocrinology* 95, 174-178 (1974).
- Katzenellenbogen, B. S. *Endocrinology* 96, 289-297 (1975).
- Clark, J. H., Peck, E. J., Jr, & Anderson, J. N. *Nature* 251, 446-448 (1974).
- Capony, F. & Rochefort, H. *Molec. cell. Endocr.* 3, 233-251 (1975).
- Katzenellenbogen, B. S. & Ferguson, E. R. *Endocrinology* 97, 1-12 (1975).
- Clark, J. *et al.* in *Hormone and Antihormone Action at the Target Cell* (eds Clark, J., Klee, W., Levitzki, A., and Wolff, J.) pp. 147-169 (Dahlem Konferenzen, Berlin, 1976).
- Oka, T. & Schimke, R. T. *J. Cell Biol.* 43, 123-137 (1969).
- Toft, D. O. & O'Malley, B. W. *Endocrinology* 90, 1041-1045 (1972).
- Mester, J. & Baulieu, E. E. *Eur. J. Biochem.* 72, 405-414 (1977).
- Palmiter, R. D., Moore, P. B. & Mulvihill, E. R. *Cell* 8, 557-572 (1976).
- Clark, J. H. & Peck, E. J. *Jr Nature* 260, 635-637 (1976).
- Lebeau, M. C., Massol, N. & Baulieu, E. E. *Eur. J. Biochem.* 36, 294-300 (1973).
- Harper, M. J. K. & Walpole, A. L. *J. Reprod. Fert.* 42, 251-258 (1967).
- Lee, A. E. *J. Endocr.* 60, 167-174 (1974).

Induction of human follicle-stimulating hormone in HeLa cells by sodium butyrate

THE ectopic production of polypeptide hormones by tumours derived from tissues that normally do not synthesise these proteins provides a means for investigating regulation of gene expression. All of the hormones ectopically synthesised by non-endocrine tumours are polypeptides or prostaglandins; however, pituitary gonadotropin production by neoplasm as opposed to chorionic gonadotropins is apparently very rare¹. Only one patient has been reported in which a bronchiogenic carcinoma produced follicle-stimulating hormone (FSH) and luteinising hormone (LH)

Table 1 Enhancement of FSH production by butyrate in HeLa cells

Subline	β -subunit of FSH antiserum FSH(ng) secreted* by 10^6 cells			Complete FSH antiserum FSH(mIU) [†] secreted* by 10^6 cells		
	Mean	Range	Proportional increase	Mean	Range	Proportional increase
HeLa ₆₅ , control	2.9	0.3–4.8	1	1.7	1.3–2.1	1
HeLa ₆₅ , 1 mM butyrate	151.7	106.1–235.8	52	6.0	5.2–6.7	3.5
HeLa ₆₅ , 5 mM butyrate	1,007.6	590.9–1590.9	347	51.0	47.7–54.5	30
HeLa ₇₁ , control	116.6	65.9–204.5	1	2.7	2.5–2.8	1
HeLa ₇₁ , 1 mM butyrate	731.7	609.8–975.6	6	16.0	14.6–17.1	6
HeLa ₇₁ , 5 mM butyrate	17,555.5	9,333.3–28,333.3	151	313.0	293.3–333.3	116

HeLa₆₅ and HeLa₇₁ cells were cultured with and without sodium butyrate for 68 h in Waymouth's medium with 10% foetal calf serum and antibiotics (penicillin 30 μ g; streptomycin 50 μ g and kanamycin 30 μ g ml⁻¹). Methods of subculturing, collecting and preparing cells for assays were as before⁴. The media and ultrasonicates of cells were tested for FSH by radioimmunoassay (RIA) using ¹²⁵I-FSH as the tracer antigen^{8–10}. Radiolabelled iodinated FSH was prepared by the Chloramine-T method and purified on polyacrylamide gel. To minimise cross-reactions with other closely related glycopolypeptides, one antiserum was raised in rabbits against the β -subunit of FSH extracted and purified from fresh frozen human pituitary glands (from Diagnostic Biochem). A second antiserum was prepared against highly purified complete human pituitary FSH (Institute of Bioendocrinology, Montreal). Radioactive antigen-antibody complexes were isolated by double-antibody precipitation technique⁸ using as the second precipitant antibody, an anti-rabbit γ globulin prepared in goats with antiserum against the β subunit and an anti-rabbit γ globulin prepared in sheep with antiserum against complete FSH. Radiolabelled precipitate was collected by centrifugation at 6,000g at 2°C and counted in a Beckman Biogamma II spectrometer. The percentage of binding of radioactive ¹²⁵I-FSH by antiserum against the β subunit of FSH was plotted against pg concentrations of purified human pituitary FSH as the standard on logit-log graph paper. The limit of sensitivity of the assay was 50 pg. The percentage binding of radioactive ¹²⁵I-FSH by antiserum against complete FSH was plotted against mIU of Second International Reference Preparation of Human Menopausal urine Gonadotropin (2nd IRP HMG) as the standard. The limit of sensitivity of the assay was 0.16 mIU. Concentrations in unknown samples were determined either from the linear standard logit-log plots or from sigmoid calibration curves on semi-logarithmic scales. Apparent differences in concentrations of FSH as measured by the two antisera are probably the result of differences in the standards used and the higher sensitivity of the radioimmunoassay with β -FSH antiserum. The results presented in the table are means of duplicate assays on three separate experiments carried out at different times. A standard of authentic FSH at seven different dilutions and a sample of human serum with a known concentration of FSH were assayed concurrently with unknown samples to ensure accuracy and reproducibility of the technique. As measured by RIA, TSH and human placental lactogen (HPL) could not be detected nor induced by sodium butyrate in these HeLa sublines.

*As measured in the medium.

[†]1 mIU equivalent to 35ng of LER 907 FSH standard (NIH).

(ref. 2). Glycopeptide hormones—FSH, LH, thyroid-stimulating hormone (TSH) and human chorionic gonadotropin (HCG)—are composed of two dissimilar subunits: an α subunit that is common to all the glycopeptide gonadotropic hormones and a β subunit unique for each hormone that confers biological specificity³. It has been shown that HeLa cells, derived over 25 yr ago from a carcinoma of the cervix, secrete HCG (refs 4,5) and the free α subunit of glycopeptide hormones⁶. Synthesis of HCG (ref. 4) and the α subunit⁶ is markedly increased by growth in medium with sodium butyrate. We report that HeLa cells also produce pituitary FSH which is induced by butyrate. The surprising synthesis of FSH by a non-pituitary cell has required validation of the radioimmunoassay (RIA) to exclude cross-reaction with other gonadotropic hormones.

Two different antisera were used; one was prepared against highly purified human pituitary β subunit of FSH and the other against the complete hormone. The anti- β FSH serum had the following cross-reactivities: FSH 100%, HCG <1%, LH <5%, TSH <2% and growth hormone (GH) <1%. A second antiserum prepared against intact human pituitary FSH had the following cross-reactivities: <0.01% human serum albumin; <2% TSH and <0.01% with LH, HCG and GH. The tracer antigen used in the

radioimmunoassay was highly purified human pituitary ¹²⁵I labelled FSH.

Table 1 shows that two well-characterised clonal lines of HeLa cells, HeLa₆₅ and HeLa₇₁ (ref. 7), produce different basal levels of FSH. Similar differences between HeLa sublines have been observed with α chain^{5,6} and HCG production⁵ and may reflect functional alterations related to the well-known karyotypic differences between these sublines. Growth of replicate cultures of HeLa₆₅ and HeLa₇₁ cells with 1 and 5 mM butyrate caused a marked increase in FSH production (Table 1). HeLa₇₁ cells produce much more FSH in the basal and butyrate-induced state than HeLa₆₅. Dilution curves for immunoreactive substances in HeLa cell media were similar to those of authentic FSH. As shown in Table 1, the correlation of FSH values obtained by β -FSH and complete FSH assays are reasonably close except for HeLa₆₅ control where low concentrations of FSH may affect the accuracy of the assay. Differences in the apparent concentration of FSH as measured by two different antisera are probably due to the characteristics of the standards and to the greater sensitivity of β -FSH assay. Table 2 compares intracellular FSH with that secreted into the medium during 48 h of growth in medium without and with sodium butyrate. Approximately 90% of

Table 2 Comparison of intra- and extracellular levels of FSH in HeLa cells grown without and with sodium butyrate

Subline	FSH levels			
	Intracellular ng per 10^6 cells		Extracellular ng per 10^6 cells	
		Proportional increase		Proportional increase
HeLa ₆₅ , control, 48 h	0.2	1	4.6	1
HeLa ₆₅ , 5mM butyrate, 48 h	3.8	19	47.1	10.2
HeLa ₇₁ , control, 48 h	12.2	1	391.3	1
HeLa ₇₁ , 5mM butyrate, 48 h	66.7	5.5	1,666.6	4.3

FSH was measured by RIA as described in Table 1. The values are means of quadruplicates that varied by less than 15%.

Table 3 Induction of FSH in HeLa₆₅ cells by hydroxyurea and its synergism with sodium butyrate

Concentration of hydroxyurea (mM)	FSH levels in HeLa ₆₅ cultures			
	Grown for 96 h without butyrate		Grown for 96 h with 1 mM butyrate	
	ng per 10 ⁶ cells	Proportional increase	ng per 10 ⁶ cells	Proportional increase
0.00	23.8	1.0	69.7	2.9
0.01	33.1	1.4	115.1	4.8
0.05	36.0	1.5	125.0	5.3
0.25	80.8	3.4	310.3	13.0
1.00	416.7	17.5	736.8	31.0

FSH secreted in medium was determined by RIA as described in Table 1. The values are means of duplicates that varied by less than 10%.

the hormone synthesised is secreted into medium. Differences in the base-level production of FSH and the proportional increase mediated by butyrate as shown in Tables 1, 2 and 3, may be related to confluence of the culture and to the duration of growth with inducer.

Sodium butyrate has been shown to inhibit DNA synthesis in HeLa₆₅ cells¹¹. Recent studies have shown that other well-characterised inhibitors of DNA synthesis that are used as anti-neoplastic agents—hydroxyurea, methotrexate, and 1- β -D-arabinofuranosylcytosine—also increase HCG synthesis¹¹. For these reasons hydroxyurea which is a specific inhibitor of DNA but not RNA synthesis in HeLa₆₅ cells was studied for its capacity to induce FSH synthesis in HeLa₆₅ cells and to potentiate the effects of sodium butyrate (Table 3). Hydroxyurea stimulated FSH production and was synergistic to sodium butyrate (Table 3). Neither butyrate nor hydroxyurea when added to the radioimmunoassay affected measurements of FSH.

HeLa cells produce several proteins normally found in trophoblasts; these include the placental forms of alkaline phosphatase^{12,13}, ferritin^{14,15}, aldolase¹⁶, α -chains of glycopeptide hormones^{5,6}, and HCG^{4,5}. The availability of specific antisera against the human pituitary β subunit and complete FSH permitted measurement of this glycopeptide in the presence of excess α chains and HCG. The production of a presumptive pituitary gonadotropin by a non-pituitary cell is unexpected and suggests that the genome of HeLa cells is derepressed for highly differentiated gene products as well as oncofetal proteins. Butyrate, certain other aliphatic monocarboxylates and some antineoplastic drugs that inhibit DNA but not RNA synthesis, increase the activity of oncofetal alkaline phosphatase¹⁷ and HCG synthesis^{4,11}. Our finding that butyrate and hydroxyurea also stimulate FSH production suggests that inhibition of DNA synthesis or cell multiplication may have a role in modulating gene expression. Little is known about the mechanism by which specialised synthetic programmes are coupled to inhibition of DNA synthesis. The acquisition of a differentiated phenotype, however, is preceded by inhibition of cell division in several embryonic tissues^{18,19}. Studies on regulation of glycopeptide hormone production in HeLa cells may help elucidate aspects of gene expression in normal as well as malignant cells.

This work was supported by the US PHS. We thank Adriana Rukenstein for assistance.

NIMAI K. GHOSH
RODY P. COX

Division of Human Genetics,
Departments of Medicine and Pharmacology,
New York University Medical Center,
New York, New York 10016

Received 18 February; accepted 30 March 1977.

¹ Odell, W. D. & Wolfson, A. in *Cancer* 3, (ed., Becker F. F.) 81–97 (Academic, New York, 1975).

² Fauman, C., Colwell, J. A., Ryan, R. J., Hershman, J. M. & Shields, T. W. *New Engl. J. Med.* 227, 1395–1397 (1967).

³ Pierce, J. D. *Endocrinology* 89, 1331–1344 (1971).

⁴ Ghosh, N. K. & Cox, R. P. *Nature* 259, 416–417 (1976).

⁵ Lieblich, J. M., Weintraub, B. D., Rosen, S. W., Ghosh, N. K. & Cox, R. P. *Nature* 265, 746 (1977).

⁶ Lieblich, J. M., Weintraub, B. D., Rosen, S. W., Chou, J. Y. & Robinson, J. C. *Nature* 260, 530–532 (1976).

⁷ Bottomley, R. H., Trainer, A. L. & Griffin, M. J. *J. Cell Biol.* 41, 806–815 (1969).

⁸ Midgley, A. R., Jr. *Endocrinology* 79, 10–18 (1966).

⁹ Saxena, B. B., Demura, H., Gandy, H. M. & Peterson, R. E. *J. clin. Endocrinol. Metabol.* 28, 519–534 (1968).

¹⁰ Rodbard, D., Rayford, P. L., Cooper, J. A. & Ross, G. T. *J. clin. Endocrinol. Metabol.* 28, 1412–1418 (1968).

¹¹ Ghosh, N. K., Rukenstein, A. R. & Cox, R. P. *Fedn Proc.* 36, 910 (1977).

¹² Elson, N. A. & Cox, R. P. *Biochem Genet.* 3, 549–561 (1969).

¹³ Singer, R. M. & Fishman, W. H. in *Iszymes: Developmental Biology* 3, (ed. Markert, C. L.) 753–774 (Academic, New York, 1975).

¹⁴ Richter, G. W. *Nature* 207, 616–618 (1965).

¹⁵ Drysdale, J. W. & Singer, R. M. *Cancer Res.* 34, 3352–3354 (1974).

¹⁶ Gliksmann, R., Ghosh, N. K. & Cox, R. P. *Enzyme* (in the press).

¹⁷ Deutsch, S. I., Ghosh, N. K. & Cox, R. P. *Clin. Res.* 24, 641A (1976).

¹⁸ Holtzer, H., Weintraub, H., Mayne, R. & Mochan, B. *Curr. Top. Devl Biol.* 7, 229–256 (Academic, New York, 1972).

¹⁹ Holtzer, H. in *Cell Differentiation* (eds Schjeide, O. & DeVellis, J.) 476–503 (Van Nostrand-Reinhold, Princeton, 1970).

Parallel expression of new idiotypes on T and B cells

THE structural nature of the antigen receptor on thymus-derived (T) cells is as yet unresolved^{1–4}. It has been clearly demonstrated that antigen receptors on bone marrow-derived (B) cells are immunoglobulin (Ig) molecules which are similar to the antibodies secreted by their progeny^{5–7}. This is further reflected in the presence of common variable region determinants present on Ig receptors and on antibodies produced by B cells belonging to the same clone^{8,9}. These variable region determinants are called idiotypes¹⁰. We report here the use of anti-idiotypic antibodies to determine whether receptors on T and B cells specific for the hapten phosphorylcholine (PC) share idiotypic determinants. The majority of PC-specific B cells in BALB/c mice have receptors bearing an idio type characteristic of the myeloma protein TEPC 15 (T15) (ref. 8). To determine whether receptors on PC-specific T cells also reflect this 'idiotypic homogeneity', we have induced PC-specific helper T cells and attempted to inhibit their activity using anti-T15 antibodies. Our results indicate that receptors on PC-specific T and B cells share similar idiotypes.

To obtain hapten-specific T cells, we immunised BALB/c mice with either PC-conjugated MOPC 315 (PC₃-M315) (ref. 11) or dinitrophenyl (DNP)-conjugated mouse immunoglobulins⁷ (DNP₉-MIg) as previously described¹². At least 6 weeks after priming, splenic T cells were purified by nylon wool filtration¹³. Bovine serum albumin (BSA)-primed B cells were obtained from BALB/c mice immunised as previously described¹². At least 6 weeks after priming, spleen cells from these mice were treated with anti-Thy 1.2 serum and complement.

The PC-M315 and DNP-MIg-primed splenic T cell populations were assayed for helper activity in an adoptive transfer system with BSA-primed B cells. Either 50 μ g of aqueous PC-conjugated BSA (PC₂₈-BSA) (ref. 11) or 50 μ g of aqueous DNP-conjugated BSA (DNP₄₅-BSA) (ref. 7) was given intravenously with the cell inoculum. The mice

Table 1 Demonstration of PC- and DNP-specific helper T cells

BSA-primed† B cells	No. of cells transferred ($\times 10^6$)* PC-primed‡ T cells	DNP-primed T cells	Antigen	Indirect anti-BSA§ PFC per spleen ($\times 10^3$)
10	—	—	PC ₂₈ -BSA	14 (11–18)
—	15	—	PC ₂₈ -BSA	2 (1–3)
—	—	15	PC ₂₈ -BSA	0.5 (0.4–0.6)
10	—	—	DNP ₄₅ -BSA	13 (10–17)
—	15	—	DNP ₄₅ -BSA	2 (1–3)
—	—	15	DNP ₄₅ -BSA	0.5 (0.3–0.6)
10	15	—	PC ₂₈ -BSA	297 (266–331)
10	15	—	DNP ₄₅ -BSA	33 (25–43)
10	—	15	DNP ₄₅ -BSA	185 (163–210)
10	—	15	PC ₂₈ -BSA	36 (31–42)

*Cells were transferred into 600 R irradiated BALB/c mice. Animals received 50 μ g of aqueous antigen simultaneously with the cells and were killed on day 8 post cell transfer.

†Spleen cells from BSA-primed mice were treated with anti-Thy 1.2 antiserum and complement.

‡Mice were primed with either PC₂₈-M315 or DNP₄₅-M1g. T cells were purified by nylon wool filtration¹³. Both T cell populations contained > 90% T cells and 1% Ig bearing cells as assessed by immunofluorescent staining.

§Geometric mean of the responses of four or five animals per group. Numbers in parenthesis represent one standard error about the mean. The direct anti-BSA PFC response in all groups was less than 5% of the total response. Indirect anti-BSA PFC were developed using a polyvalent rabbit anti-mouse immunoglobulin antiserum.

were killed 8 d later and their spleens assayed for anti-BSA plaque-forming cells¹⁴ (PFC) using BSA-conjugated sheep erythrocytes¹⁵ as indicator cells.

Transfer of 15×10^6 PC-primed T cells together with 10×10^6 BSA-primed B cells and PC₂₈-BSA, resulted in a response of 29,700 indirect anti-BSA PFC per spleen (Table 1). This response is 19-fold higher than the background response calculated by summing the responses obtained on transfer of the same cell populations separately, that is, 1,600 anti-BSA PFC. When the same cell populations were transferred with DNP₄₅-BSA as antigen, the resulting anti-BSA PFC response was only two-fold above background (Table 1). Similarly, transfer of 15×10^6 DNP-primed T cells with 10×10^6 BSA-primed B cells resulted in an anti-BSA PFC response 12-fold above background with DNP₄₅-BSA as antigen and only two-fold higher than background when PC₂₈-BSA was used as antigen (Table 1).

To assess whether the helper activity of PC-specific T cells could be inhibited with anti-T15 antibodies, irradiated BALB/c mice were injected with anti-T15 antibodies 24 h before and at the time of, transfer of PC-primed T cells and BSA-primed B cells. Antigen (50 μ g of PC₂₈-BSA) was given 16 h after cell transfer. The animals were killed 8 d after antigen administration and their spleens assayed for anti-BSA PFC.

The data in Table 2 show that anti-T15 antibodies com-

pletely inhibit PC-specific helper activity. When 10×10^6 PC-primed T cells were transferred with 5×10^6 BSA-primed B cells and PC₂₈-BSA into conventional irradiated recipients, the resulting anti-BSA PFC response was six-fold higher than background, that is, 9,100 against 1,500 anti-BSA PFC per spleen respectively. Treatment of the recipients with anti-T15 antibodies inhibited the anti-BSA PFC response to less than 1% of the control response, that is, 1,400 anti-BSA PFC per spleen. The specificity of inhibition was established by assessing the effect of anti-T15 antibodies on the expression of DNP-specific helper activity. When 10×10^6 DNP-primed T cells were transferred with 5×10^6 BSA-primed B cells and DNP₄₅-BSA, the resulting anti-BSA PFC response was sevenfold above background, that is, 8,600 against 1,200 anti-BSA PFC spleen. Treatment of DNP-primed T cell recipients with anti-T15 antibodies did not significantly affect the anti-BSA PFC response which remained ninefold higher than background (Table 2).

The irradiated recipients used in these experiments contained high levels of T15-bearing Ig with demonstrable anti-PC activity¹⁶. It is conceivable that on transfer into irradiated recipients, PC-specific T cells bind PC₂₈-BSA which in turn picks up circulating T15 positive anti-PC antibodies. Thus, PC-specific T cells obtained from conventional BALB/c mice could bear T15-negative receptors and their helper activity might still be inhibited by anti-T15

Table 2 Inhibition of PC-specific helper activity with anti-T15 antibodies

BSA-primed† B cells	No. of cells transferred ($\times 10^6$)* PC-primed‡ T cells	DNP-primed T cells	Antigen	Anti-T15§	Indirect anti-BSA PFC per spleen ($\times 10^3$)¶	% of control**
5	—	—	PC ₂₈ -BSA	—	8 (6–9)	—
—	10	—	PC ₂₈ -BSA	—	7 (4–12)	—
5	10	—	PC ₂₈ -BSA	—	91 (74–112)	100
5	10	—	PC ₂₈ -BSA	+	14 (10–19)	< 1
5	—	—	DNP ₄₅ -BSA	—	9 (7–14)	—
—	—	10	DNP ₄₅ -BSA	—	3 (2–4)	—
5	—	10	DNP ₄₅ -BSA	—	86 (76–97)	100
5	—	10	DNP ₄₅ -BSA	+	106 (90–125)	127

*Cells were transferred intravenously into 350 R irradiated BALB/c mice. Animals received 50 μ g of aqueous antigen intravenously, 16 h after cell transfer, and were killed on day 8 post antigen administration.

†See legend for Table 1.

‡See legend for Table 1.

§0.2 ml of a 1/2 dilution of A/J anti-T15 antiserum was administered intraperitoneally 16 h prior to, and at the time of, cell transfer. The idiotype specificity of this antiserum has been reported elsewhere^{17,18}.

¶Geometric means of the responses of three to six animals per group (all groups with T+B cells contained six animals). The direct anti-BSA PFC response in all groups was less than 5% of the total response. Indirect anti-BSA PFC were developed using a polyvalent rabbit anti-mouse immunoglobulin antiserum.

**% of control response was calculated as follows: Inhibition of PC-specific helper activity: $[14 - (8 + 7)]/[91 - (8 + 7)]$ where (8 + 7) is the background anti-BSA PFC response obtained on transfer of T or B cells alone with PC₂₈-BSA. Similarly, inhibition of DNP-specific helper activity $[106 - (9 + 3)]/[86 - (9 + 3)]$.

Table 3 Failure of anti-T15 antibodies to inhibit PC-specific helper activity induced in T15 negative BALB/c mice

BSA-primed† B cells	No. of cells transferred ($\times 10^6$)* PC-primed T cell‡ T15+	T15-	Antigen	Anti-T15§	Indirect anti-BSA¶ PFC per spleen ($\times 10^3$)	% of control**
5	—	—	PC ₂₈ -BSA	—	28 (25-31)	—
—	7.5	—	PC ₂₈ -BSA	—	2 (1.9-2.3)	—
5	7.5	—	PC ₂₈ -BSA	—	168 (141-200)	100
5	7.5	—	PC ₂₈ -BSA	+	18 (15-21)	< 0.7 ^a
5	3.8	—	PC ₂₈ -BSA	—	133 (116-154)	100
5	3.8	—	PC ₂₈ -BSA	+	31 (24-39)	1 ^b
5	—	—	PC ₂₈ -BSA	—	13 (10-17)	—
—	—	10	PC ₂₈ -BSA	—	2 (1.5-2.8)	—
5	—	10	PC ₂₈ -BSA	—	94 (78-113)	100
5	—	10	PC ₂₈ -BSA	+	111 (92-124)	122 ^c

*See legend for Table 2.

†See legend for Table 1.

‡Splenic T cells were isolated from either conventional T15-positive (T15⁺) BALB/c mice which contained 40-80 $\mu\text{g ml}^{-1}$ of circulating Ig bearing the T15 idiotype or from T15-negative (T15⁻) BALB/c mice which had been neonatally suppressed with 0.1 ml of A/J anti-T15 antiserum and contained < 2.5 $\mu\text{g ml}^{-1}$ of circulating Ig bearing the T15 idiotype. Both types of donor were primed with PC₂₈-M315. In both cases isolation of splenic T cells resulted in lymphocyte populations which contained > 90% T cells and 1% Ig bearing cells as assessed by immunofluorescence staining.

§See legend for Table 2.

¶See legend for Table 2.

**See legend for Table 2 for explanation of calculations: a, $(18-30)/(168-30) = < 1/138 = 0.7\%$; b, $(31-30)/(133-30) = 1/103 = 1\%$; c, $(111-15)/(94-15) = 122\%$.

antibodies.

To test this possibility we took advantage of the observation that receptors on PC-specific precursor B cells in BALB/c mice neonatally suppressed with anti-T15 antibodies bear T15-negative idiotypes^{17,18}. If neonatal idiotype suppression induces a parallel change in receptors expressed by PC-specific T cells, their helper activity should not be inhibited by anti-T15 antibodies, unless they adsorb T15-positive anti-PC antibodies naturally present in the conventional BALB/c recipients. The results in Table 3 show that when either 7.5×10^6 or 3.8×10^6 PC-primed T cells from T15-positive donors were transferred with 5×10^6 BSA-primed B cells, the resulting anti-BSA PFC responses obtained were sixfold higher than background. Again, these responses were completely abolished by treatment of recipients with anti-T15 antibodies, that is, less than 1% of the control response (Table 3). Comparable helper activity was obtained on transfer of 10×10^6 PC-primed T cells from T15-negative donors. Treatment of recipients with anti-T15 antibodies, however, did not significantly affect the anti-BSA PFC response which remained sevenfold above background, that is, 122% of the control response (Table 3). We conclude that the target of anti-T15 antibodies is not a molecule passively adsorbed on the T cell surface either directly or through antigen bridge; it is the functional receptor present on PC-specific T cells which bears the T15 idiotype.

These studies demonstrate a parallel behaviour between antigen receptors on PC-specific T and B cells. As has been previously shown, antibodies directed towards the T15 idiotype specifically inhibit the function of PC-specific B cells from T15-positive BALB/c mice⁸. Similarly, the helper activity of PC-specific T cells from T15 positive mice is also inhibited by anti-T15 antibodies. Furthermore, PC-specific T and B cells from neonatally suppressed mice bear receptors which lack the T15 idiotype. These results suggest (1) that the T15 idiotype is present in the repertoire of PC-specific T and B cells and (2) that neonatal suppression of this idiotype induces the expression of T15-negative receptors in both lymphocyte lineages.

Consistent with these findings is the recent work of Binz and Wigzell which demonstrated that anti-idiotypic antibodies prepared against alloantibodies specifically removed the reactivity of T cells to the same alloantigen¹⁹. In addition, Eichmann and Rajewsky²⁰ and Black *et al.*²¹ demonstrated that anti-idiotypic antibodies were able to

induce priming of both B and T cells with specificity for the same antigen. In addition to the present report, these results strongly suggest that the specificity of antigen recognition by B and T cells is very similar and that this specificity is imparted by structurally similar molecules. It has been reported, however, that T cells contain and secrete antigen-specific factors bearing markers which are *Ir* gene products^{22,23}. In addition, products of the major histocompatibility complex restrict the function of T lymphocytes of any specificity²⁴⁻²⁷. It is possible, therefore, that the V-region structures of receptors on T and B cells specific for the same antigen, bear similar idiotypes associated with structurally different molecules.

We thank Rosemarie Lang, John Bews and Heidi Amstutz for technical assistance.

MICHAEL H. JULIUS*
HUMBERTO COSENZA
ANDRE A. AUGUSTIN

Basel Institute for Immunology,
Grenzacherstrasse 487,
Postfach 4005,
Basel 5, Switzerland

Received 15 February; accepted 5 April 1977.

*To whom correspondence should be addressed.

- Vitetta, E., Bianco, C., Nussenzweig, V. & Uhr, J. J. *exp. Med.* **136**, 81-93 (1972).
- Marchalonis, J. J. & Cone, R. E. *Transplantation Rev.* **14**, 3-49 (1973).
- Rabellino, E., Colon, S., Grey, H. M. & Unanue, E. R. *J. exp. Med.* **133**, 156-167 (1971).
- Roelants, G. E., Forni, L. & Pernis, B. J. *exp. Med.* **137**, 1060-1077 (1973).
- Walters, C. S. & Wigzell, H. *J. exp. Med.* **132**, 1233-1242 (1970).
- Jones, P. P., Craig, S. W., Cebra, J. J. & Herzenberg, L. A. *J. exp. Med.* **140**, 452-469 (1974).
- Julius, M. H. & Herzenberg, L. A. *J. exp. Med.* **140**, 904-920 (1974).
- Cosenza, H. & Kohler, H. *Proc. natn. Acad. Sci. U.S.A.* **69**, 2701-2705 (1972).
- Clafin, J. L., Lieberman, R. & Davie, J. M. *J. exp. Med.* **139**, 58-73 (1974).
- Odin, J. & Michel, M. C. R. *Acad. Sci. (Paris)* **257**, 805-811 (1963).
- Chesbro, B. & Metzger, H. *Biochemistry* **11**, 766-771 (1972).
- Cosenza, H., Augustin, A. & Julius, M. H. *C.S.H.S.Q.B.* **41** (in the press).
- Julius, M. H., Simpson, E. & Herzenberg, L. A. *Eur. J. Immun.* **3**, 645-649 (1973).
- Cunningham, A. J. & Szenberg, A. *Immunology* **14**, 599-600 (1968).
- Gold, E. R. & Fudenberg, H. H. *J. Immun.* **99**, 859-866 (1967).
- Lieberman, R., Potter, M., Mushinski, E. B., Humphrey, W. & Rudikoff, S. *J. exp. Med.* **139**, 983-1001 (1974).
- Cosenza, H., Augustin, A. A. & Julius, M. H. *Eur. J. Immun.* (in the press).
- Augustin, A. & Cosenza, H. *Eur. J. Immun.* **6**, 497-501 (1976).
- Binz, H. & Wigzell, H. *J. exp. Med.* **142**, 197-211 (1975).
- Eichmann, K. & Rajewsky, K. *Eur. J. Immun.* **5**, 611-666 (1975).
- Black, S. J., Hammerling, G. H., Berek, C., Rajewsky, K. & Eichmann, K. *J. exp. Med.* **143**, 846-860 (1976).
- Tada, T., Okumura, K. & Taniguchi, M. *J. Immun.* **111**, 952-961 (1973).
- Munro, A. J., Taussig, M. J., Campbell, R., Williams, H. & Lawson, Y. J. *exp. Med.* **140**, 1579-1587 (1974).
- Doherty, P. C., Bianden, R. V. & Zinkernagel, R. M. *Transplantation Rev.* **29**, 89-124 (1976).
- Shearer, G. M., Rehn, T. G. & Schmitt-Verhulst, A. M. *Transplantation Rev.* **29**, 222-248 (1976).
- Miller, J. F. A. P., Vadas, M. A., Whitelaw, A. & Gamble, J. *Proc. natn. Acad. Sci. U.S.A.* **72**, 5095-5098 (1975).
- von Boehmer, H. & Sprent, J. *Transplantation Rev.* **29**, 3-23 (1976).

Failure to demonstrate allotype-specific helper cells for antibody production in the rat

THE way in which the immune response is regulated is poorly understood but network theories, based on idio-anti-idiotypic interactions have been widely discussed¹. It has been suggested^{2,3} that in addition to the idio-anti-idiotypic network an allotype network also exists. This hypothesis rests on the demonstration³ that in a particular mouse strain, carrier-primed T cells from Ig 1-a homozygotes collaborated in a secondary anti-DNP (dinitrophenol) response with Ig 1-a allotype, but not Ig 1-b allotype, B cells from DNP-primed 1-a/1-b heterozygotes. In attempting to understand the regulation of the immune response it is clearly essential to establish whether the heavy chain allotype specificity of helper T cells shown by these experiments is common to all allotypes and is not a particular feature of the system studied. Here we describe experiments in which we were unable to demonstrate allotype-specific helper cells for antibody production in the rat.

T cells and DNP/BGG were injected intravenously into irradiated PVG/c homozygotes and the number of 1-a allotype anti-DNP plaque-forming cells per recipient spleen was determined using an autoradiographic plaque assay⁷.

The results show that in the absence of helper T cells hapten-primed B cells on transfer to irradiated recipients respond poorly to challenge with the priming antigen (Table 1). In contrast a greatly augmented B cell response is obtained when carrier-primed T cells are provided even if these derive from donors of the opposite light chain allotype. (The variation between the magnitude of augmentation in the three experiments is not statistically significant being largely a reflection of the wide variation in the small response produced by the purified B cell populations transferred.) Thus the data give no indication of allotype specificity of T cell help in this system. Because the light chain allotype is present on all classes of rat immunoglobulin the results do not exclude the possibility that allotype-specific helper cells exist for some classes but not others. Nor is it possible to rule out the existence of a cell type that regulates the expression of the two light chain allotypes in 1-a/1-b heterozygotes. It is clear, however, that in the rat carrier-primed T cells and hapten-primed B

Table 1 Collaboration between T cells from 1-b homozygote donors and 1-a allotype B cells

Experiment no.	TDL transferred $\times 10^{-6}$		No. of 1-b/1-b recipients†	1-a anti-DNP PFC per spleen (mean and range)	Collaborative increase§
	T cells* (1-b/1-b)	B† cells			
	7.4	0	3	0	
1	0	2.9 (1-a/1-b)	2	713 (285-1,140)	39
	7.4	2.9 (1-a/1-b)	2	28,070 (27,640-28,500)	
	7.4	0	3	0	
2	0	2.9 (1-a/1-b)	2	171 (114-228)	131
	7.4	2.9 (1-a/1-b)	2	22,340 (18,580-26,100)	
	7.4	0	3	0	
3	0	2.7 (1-a/1-a)	2	228 (0-456)	259
	7.4	2.7 (1-a/1-a)	2	59,050 (49,470-68,620)	
	7.4	0	3	0	

*PVG/c TDL depleted of B cells from rats primed with DNP₂₅BGG 7-12 weeks previously.

†B cells selected by FACS from TDL of DNP₂₅BGG primed (PVG/1-a $\times$ PVG/c)F₁ (first two experiments) or PVG/1-a homozygote (third experiment) rats.

‡Recipients were PVG/c rats given 500 rad (first two experiments) or 660 rad (third experiment) 6 h before cell transfer and challenge.

§Collaborative increase refers to the factor by which the 1-a PFC response is augmented by the addition of the carrier-primed T cells.

To investigate the generality of the findings of Herzenberg *et al.*³ we examined the allotype specificity of helper T cells using the rat light chain allotype marker⁴. B and T cells from appropriate donors, primed several weeks earlier with DNP-bovine γ globulin (DNP-BGG), were injected intravenously together with DNP-BGG into irradiated recipients. The splenic anti-DNP plaque-forming cell response was assayed 1 week later. Thoracic duct lymphocytes (TDL) from PVG/1-a and (PVG/1-a $\times$ PVG/c)F₁ rats were used as a source of B cells. PVG/1-a is a new strain, congenic with PVG/c, but carrying the 1-a light chain allotype. Heterozygotes were obtained from the sixteenth backcross and homozygotes from an incross of this generation. T cells were obtained from TDL of PVG/c homozygotes. B and T cell donors were primed intraperitoneally with alum-precipitated DNP-BGG and 2×10^9 Pertussis organisms 6-12 weeks before use. B cells were labelled by incubation with rabbit F(ab')₂-anti-rat Fab followed by fluoresceinated horse-anti-rabbit Fab and isolated using a fluorescence-activated cell sorter⁵. T cells were purified by rosetting TDL with rabbit-anti-rat F(ab')₂ coated sheep red blood cells and subsequent fractionation on an Isopaque-Ficoll gradient⁶. Various mixtures (see Table 1) of B cells,

cells derived from donors of different allotype can collaborate efficiently.

Elson and Taylor⁸ using the same allotypic marker as Herzenberg *et al.*³, but with a different congenic strain combination, found no evidence of allotype restriction of helper T cells. These findings taken together with our own suggest that the contrary findings of Herzenberg *et al.* may be dependent in some way on the particular mouse strain used by these workers.

We thank Dr S. V. Hunt for the gift of rats of the new strain developed by him, Dr A. F. Williams for immunoabsorbent purified antibodies, Miss L. Hackfath for technical assistance and to Mrs J. Shepherd for typing the manuscript.

D. W. MASON
S. J. SIMMONDS
R. A. H. WHITE

MRC Cellular Immunology Unit,
Sir William Dunn School of Pathology,
Oxford University, Oxford, UK

Received 15 February; accepted 19 April 1977.

¹ Jerne, N. K. *Ann. Immun. (Inst. Pasteur)* 125C, 273 (1974).

² Raff, R. *Nature* 265, 205 (1977).

- ³ Herzenberg, L. A. *et al.* *J. exp. Med.* **144**, 330 (1976).
⁴ Armerding, D. *Eur. J. Immun.* **1**, 39 (1971).
⁵ Hulet, H. R., Bonner, W. A., Sweet, R. G. & Herzenberg, L. A. *Clin. Chem.* **19**, 813 (1973).
⁶ Parish, C. R. & Hayward, J. A. *Proc. R. Soc. B187*, 65 (1974).
⁷ Mason, D. W. *J. Immun. Meth.* **10**, 301 (1976).
⁸ Elson, C. J. & Taylor, R. B. *Immunology* **28**, 543 (1975).

T-cell mediated immunity towards antigen(s) on isologous erythrocytes

SPLEENS of normal mice contain large numbers of cells which produce antibody against antigenic determinants on their own erythrocytes^{1,2}. To demonstrate this autoimmune response, the mouse erythrocytes (MRBC) must be treated with proteolytic enzymes, such as trypsin or bromelain, in order to reveal hidden autoantigen(s)³. Treatment of mice with anti-lymphocyte serum (ALS) causes a 20-fold rise in the number of cells producing antibody to bromelain-treated mouse erythrocytes (BrMRBC) (ref. 2). Based on this observation, Cunningham has proposed that the number of cells making anti-BrMRBC antibody is controlled by an active, ongoing, T-cell induced suppression³. We report here that the culturing of normal mouse spleen cells *in vitro* not only allows the development of cells producing antibody to BrMRBCs, but also T cells which mediate, on transfer, delayed-type hypersensitivity (DTH).

Normal C57B1/6J (6–8-week-old) mouse spleen cells were cultured in Marbrook chambers⁴ at a concentration of 1.5×10^7 ml⁻¹ in CMRL 1066 medium, supplemented with 10 ml l⁻¹ each of 100-times concentrated MEM non-essential amino acids, L-glutamine, sodium pyruvate and 17% heat-inactivated foetal calf serum. The preparation of BrMRBC and the plaque-forming cell (PFC) assays were carried out as described by Cunningham¹. The separation of immunoglobulin-bearing cells, the transfer of cells into the footpads of mice, and the measurement of DTH reactions have been described in detail elsewhere^{5,6}.

When suspensions of normal spleen cells are cultured *in vitro*, a substantial anti-BrMRBC PFC response develops within 2 d (Table 1). The cultured spleen cells also induce DTH reactions when they are injected with BrMRBC into the footpads of normal mice (Table 1). In contrast to the antibody response, which peaks at day 2, the largest DTH reactions are obtained with cells taken after 4 d in culture and at a time when the antibody response is declining.

The T-cell dependence of the DTH reaction was demonstrated by treating cultured spleen cells with anti-Thy 1.2 serum and complement. Whereas untreated cultured spleen cells or cells treated with normal serum and complement induced large DTH reactions, cells treated with anti-Thy 1.2 serum and complement produced only a background footpad swelling (Table 2). Furthermore, depleting cultured

Table 2 T-cell dependence of the delayed-type hypersensitivity reaction against BrMRBC

Treatment of cultured spleen cells*	BrMRBC added	DTH reaction
None	+	17.0±0.15
None	—	4.3±0.21
Anti-Thy 1.2+C	+	5.0±0.22
Normal serum + C	+	17.5±1.7
Depletion of Ig ⁺ cells	+	18.0±0.8

*Spleen cells were cultured for 4 d, collected, washed and the red cells and dead cells removed by centrifugation on Isopaque/Ficoll¹⁸. Depletion of immunoglobulin-bearing cells and treatment with anti-Thy 1.2 serum are described in detail elsewhere^{5,6}. The efficiency of depleting Ig-bearing cells has been shown⁶ to be > 97%. 10⁷ cultured spleen cells with 10⁸ BrMRBC were injected in volumes of 15 µl into the footpads of normal mice. The increase in footpad swelling is expressed in units of 0.01 mm±s.e. Background footpad swellings ranging of 3.5–4.5 were caused by the trauma of injection.

spleen cells of immunoglobulin-bearing cells before cell transfer did not affect the level of DTH obtained (Table 2). The specificity of the DTH reactions was studied by transferring, into the footpads of normal mice, mixtures of cultured cells and various antigens (Table 3). Only with BrMRBC, sheep red blood cells (SRBC) or bromelain-treated SRBC, but not with several other antigens tested, including untreated MRBC (Table 3), were DTH reactions elicited. BrMRBC and SRBC also cross react at the antibody level⁷.

These findings raise the question of how this cell-mediated autoimmune response is maintained *in vivo* at a relatively low level. Cunningham³ has proposed that suppressor T cells control the number of PFC against BrMRBC. A similar mechanism may also control the T-cell mediated immunity (CMI). This view is supported by our findings that suppressor T cells are induced with the establishment

Table 3 Specificity of the delayed-type hypersensitivity reaction induced by cultured spleen cells

Antigen	Cultured cells†	DTH reaction
None	+	4.1±0.13
BrMRBC*	+	18.5±1.2
BrMRBC	—	4.2±0.2
MRBC	+	4.16±0.13
SRBC	+	15.1±0.59
BrSRBC*	+	15.5±1.06
HRBC	+	4.3±0.54
BrHRBC*	+	6.3±0.72
GRBC	+	4.1±0.54
BrGRBC*	+	4.2±0.72
KLH‡	+	3.5±0.23

*Bromelain-treated mouse (M), sheep (S), horse (H) or goose (G) erythrocytes.

†Spleen cells were cultured for 4 d, collected, washed and the red cells and dead cells removed by centrifugation on Isopaque/Ficoll. 10⁷ recovered viable cells with antigen were injected in volumes of 15 µl into the footpads of normal mice. DTH reactions were read 24 h later. Additional experimental details as for Table 1.

‡50 µg Keyhole limpet haemocyanin (KLH) was injected with cultured spleen cells.

Table 1 Development of T cells which mediate delayed-type hypersensitivity against BrMRBC

Days in culture	DTH reactions against BrMRBC*	Plaque-forming cells against BrMRBC†
0	4.2±0.21†	154±54
1	5.5±0.23	960±120
2	10.1±0.95	8,620±710
3	14.8±0.49	6,640±509
4	19.5±0.6	2,010±346

*Cultured spleen cells were collected, washed and the red cells and dead cells removed by centrifugation on Isopaque/Ficoll¹⁸. 10⁷ recovered viable cells with 10⁸ BrMRBC were injected in volumes of 15 µl into the footpads of normal mice. The increase in footpad swelling is expressed in units of 0.01 mm±s.e.

†Background footpad swellings ranging between 3.5 and 4.5 were caused by the trauma of injection.

‡Plaques per 10⁷ viable recovered cells±s.e.

of different, and opposing, types of immunity. Thus, T cells induced under conditions of humoral immunity can suppress CMI responses⁸, whilst CMI cells can repress the induction of humoral immunity^{8,9}. BrMRBC antigens may induce a balanced state of CMI and IgM antibody with different populations of suppressor T cells controlling each type of immunity. If these postulates are correct, then manipulations which suppress CMI should also enhance the humoral immune response to BrMRBC. Thus, for

example, administration of ALS, which is known to selectively suppress CMI in sensitised animals¹⁰, also causes an increase in the number of PFC to BrMRBC².

Numerous other studies have shown that a wide range of self-antigens exist which are hidden on intact cells¹¹⁻¹³. Degenerating or dead cells may reveal these cryptic antigens which then stimulate both humoral and CMI responses. This autoimmunity seems harmless and may, in fact, be beneficial in removing dying or dead cells.

Furthermore, it is possible that such autoimmune responses may act as an immune surveillance mechanism that prevents the development of 'abnormal' cells such as tumour cells. Normal cells would be safe from immune attack because the self-antigens are hidden. An 'abnormal' cell that has an altered cell membrane may, however, have these cryptic sites exposed resulting in their immune destruction. Such an immune surveillance mechanism has the advantage that the immune response is primed and therefore need not adapt to new antigens on the tumour cells. Many naturally-occurring antibodies^{14,15} and cytotoxic cells^{16,17} specific to spontaneous or carcinogen-induced tumours have been found in normal mice. The antigens, against which this natural immunity is directed, have not been identified. Nonetheless, the wide-ranging differences that are known to exist between normal and tumour cell membranes are compatible with the view that natural immunity may be directed against normally hidden self-antigens that have become exposed on the tumour cells.

We thank Gail Minish, Janet Reymond and Deborah Alward for technical assistance. This work was supported by the National Cancer Institute of Canada, Ontario Heart Foundation and the MRC. I.A.R. is a fellow of the MRC of Canada.

IAN A. RAMSHAW

DAVID EIDINGER

Department of Microbiology and Immunology,
Queen's University,
Kingston, Ontario,
Canada

Received 17 January; accepted 13 April 1977.

- ¹ Cunningham, A. J. *Nature* 252, 749-751 (1974).
- ² Cunningham, A. J. *Nature* 254, 143-144 (1975).
- ³ Cunningham, A. J. *Transplantation Rev.* 31, 23-43 (1976).
- ⁴ Marbrook, J. *Lancet* ii, 1279-1281 (1967).
- ⁵ Ramshaw, I. A., Bretscher, P. A. & Parish, C. R. *Eur. J. Immun.* 6, 674-679 (1976).
- ⁶ Parish, C. R., Kirov, S. M., Bower, N. & Blanden, R. V. *Eur. J. Immun.* 4, 808-815 (1974).
- ⁷ Pages, J. & Bussard, A. E. *Nature* 257, 316-317 (1975).
- ⁸ Ramshaw, I. A., Bretscher, P. A. & Parish, C. R. *Eur. J. Immun.* (in the press).
- ⁹ Ramshaw, I. A., McKensie, I. F. C., Bretscher, P. A. & Parish, C. R. *Cell Immun.* (in the press).
- ¹⁰ Lance, E. M., Medawar, P. B. & Taub, R. N. *Adv. Immun.* 17, 1-92 (1973).
- ¹¹ Reisner, E. E. & Amos, D. B. *Transplantation* 14, 455-461 (1972).
- ¹² Rogentine, G. N. & Plocinik, B. A. *J. Immun.* 113, 848-858 (1974).
- ¹³ Rosenberg, S. A. & Rogentine, G. N. *Nature new Biol.* 239, 203-204 (1972).
- ¹⁴ Herberman, R. B. & Aoki, T. *J. exp. Med.* 136, 94-111 (1972).
- ¹⁵ Martin, S. T. & Martin, W. J. *Int. J. Cancer* 15, 658-664 (1975).
- ¹⁶ Gomard, E., Leclerc, J. C. & Levy, J. P. *Nature* 250, 671-673 (1974).
- ¹⁷ Herberman, R. B., Nunn, M. B. & Lourin, D. H. *Int. J. Cancer* 16, 216-229 (1975).
- ¹⁸ Davidson, W. F. & Parish, C. R. *J. Immun. Meth.* 7, 291-299 (1975).

Uptake of nucleosides in density-inhibited cultures of 3T3 cells

AN increase in the rate of nucleoside incorporation into acid-soluble pools is one of the earliest events seen when fibroblasts arrested in the G₁/G₀ or A state¹ are stimulated to grow by the addition of serum²⁻⁵. The stimulation of uridine uptake is preceded by a lag phase of several

minutes⁶ and is not prevented by inhibitors of protein synthesis⁷. The uptake of nucleosides by mammalian cells seems to proceed in two steps: (1) the nucleosides are transferred rapidly across the plasma membrane by a facilitated diffusion mechanism⁸ and (2) the intracellular nucleosides are phosphorylated by specific nucleoside kinases and the nucleosides formed are trapped intra-

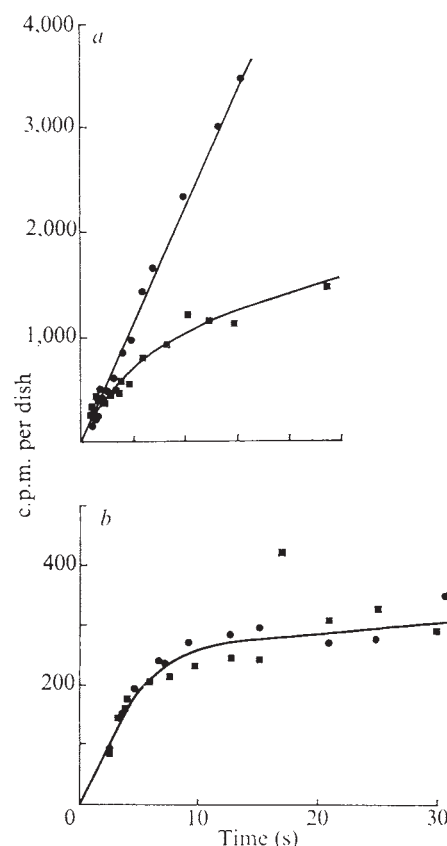


Fig. 1a, Uptake of labelled uridine by 3T3 cells as a function of time at 37 °C, in cultures previously activated by exposure to 10% foetal calf serum for 30 min. (circles) or in quiescent cells, exposed only to a serum-free medium (squares). Swiss mouse 3T3 cells¹⁴, propagated as previously described¹⁵, were subcultured into 30-mm Nunc dishes in 6% foetal calf serum; medium was changed at 3 d. Uptake activity was always assayed on confluent cells 12-15 d later. Uptake was measured by adding at zero time about 2 ml of Dulbecco modified minimal Eagles' medium (DME) containing 1.5 μM uridine, labelled radioactively as 5-³H-uridine at 1.67 Ci mmol⁻¹. At the times indicated on the abscissa, the medium was removed rapidly and the dishes were washed six times with ice-cold 0.14 M NaCl and the cultures were extracted with 1 ml of 5% trichloroacetic acid at 4 °C for 20 min. Acid-soluble pools (0.9 ml) were removed for scintillation counting with a Triton-toluene solution of phosphor. Samples for the zero-time values received the same medium and radioactivity, with the exception that it contained 1 μM NBMI, for about 2 s, before washing six times with an ice-cold solution of 1 μM NBMI in saline. The results are expressed on the ordinate as c.p.m. per dish from which the zero-time values of 281 ± 62 (s.e.m., four determinations) c.p.m. per dish for the serum-stimulated cultures, and 279 ± 55 (s.e.m., four determinations) c.p.m. per dish for the non-stimulated cultures, have been subtracted. **b**, Uptake of labelled cytosine-β-D-arabino- side as a function of time at 37 °C into 3T3 cells stimulated by serum (circles) or into non-stimulated cells (squares). Uptake was measured as for Fig. 1a, except that 5-³H-cytosine-β-D-arabino- side was at specific activity 0.5 Ci mmol⁻¹ and concentration 5 μM. The zero-time values were 89 ± 2 (s.e.m., three determinations) c.p.m. per dish for the serum-stimulated cells and 54 ± 8 (s.d. two determinations) c.p.m. per dish for the non-stimulated cultures.

cellularly⁹. Although the regulation of nucleoside uptake has been studied in some detail³⁻⁷, it is not known which of these two steps is activated by growth-promoting agents. We have investigated which step of nucleoside uptake is stimulated by serum in density-inhibited cultures of 3T3 cells. The results indicate that phosphorylation is the rate-limiting step in nucleoside uptake by quiescent cells. Growth stimulation by serum enhances the rate of phosphorylation, but apparently not that of transport.

Figure 1a shows the time course of uridine uptake by cultures that were exposed to serum for 30 min and therefore have a fully stimulated uptake⁶ compared with cultures shifted to synthetic medium alone (non-stimulated cultures). While the time course is linear in serum-activated cells, the pattern is drastically different in the non-stimulated cultures. For the first 4 or 5 s both sets display no apparent difference in uptake while afterwards the curve depicting the uptake by non-stimulated cultures levels off sharply. These results suggest that the initial rate of uridine transport is not substantially different in serum-stimulated and non-stimulated cultures, but that intracellular trapping by the kinase is much less efficient in resting cells. We also measured the time-course of uptake of cytosine- β -D-arabino-*s*ide, a nucleoside which is transported

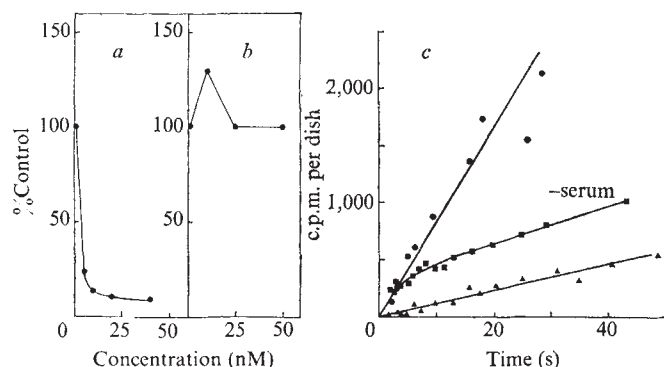


Fig. 2 *a*, Inhibition by NBMI of uridine uptake into quiescent 3T3 cells. Uridine uptake was measured as described for Fig. 1, except that the time of incubation was everywhere 6 min (at 37 °C). The cultures received NBMI at the concentration indicated on the abscissa for 10 min before the assay uptake. *b*, Effect of NBMI on the activity of uridine kinase isolated from 3T3 cells. The assay of uridine kinase was modified from that of Mizel and Wilson¹⁶. Cultures of quiescent 3T3 cells were washed with isotonic saline and scraped off the dish with a rubber policeman. The cells were pelleted, resuspended in 10 mM Tris-HCl, pH 7.5; 10 mM KCl; 2 mM MgCl₂, swollen for 10 min and disrupted in a Dounce homogeniser. The homogenate was centrifuged at 23,400g for 20 min. The incubation was performed at 37 °C in 400 μ l of solution containing 50 mM HEPES, pH 7.4; 2 mM ATP; 2 mM MgSO₄; 4 mM 3-PGA and 1 μ M 5-³H-uridine (5 Ci mmol⁻¹) and concentrations of NBMI indicated on the abscissa. The reaction was started by the addition of cell extract and stopped by boiling (3 min); samples of 100 μ l were removed at 3-min intervals from the incubation mixture. After centrifugation to remove denatured protein, 50- μ l samples were applied to DEAE filters (Whatman DE-81). The filters were washed and the radioactivity retained was measured as before¹⁶. The time course of the reaction was linear for at least 9 min of incubation. *c*, Uptake of labelled uridine as a function of time into 3T3 cells stimulated by serum (circles), or into non-stimulated cells (squares) or into non-stimulated cells treated for 30 min at 37 °C with 1.5 mM NBMI, uptake being measured in the presence of a similar concentration of NBMI (triangles). Uptake was measured as described for Fig. 1, except that the zero-time values were obtained in the presence of 50 nM NBMI, and the washing solution also contained this reduced concentration of NBMI. The zero-time values were: for the serum-stimulated cultures, 456 $\pm$ 23 (s.e.m., three determinations) c.p.m. per dish, for the non-stimulated cultures, 209 $\pm$ 50 (s.e.m., three determinations) c.p.m. per dish.

across the cell membrane¹⁰ partly by the uridine 'carrier' system (personal communication from Z. I. Cabantchik and Y. Eilam) but is phosphorylated by deoxycytidine kinase rather than by uridine kinase^{11,12}. Figure 1b shows that the initial velocity of entry of cytosine- β -D-arabino-*s*ide into serum-stimulated cells does not differ from that of the controls. This experiment reinforces the conclusion that (nucleosides are transferred rapidly across the cell membrane of mammalian cells whether they are resting or stimulated to grow.

If the biphasic shape of the uptake curve of the non-stimulated cultures shown in Fig. 1a results from a rapid transfer of nucleoside followed by a low rate of phosphorylation, it should be possible to convert such a biphasic curve into a straight line when transport is selectively inhibited, so that transport (rather than internal phosphorylation) becomes rate-limiting. NBMI (6-nitrobenzyl mercaptosine), a highly potent and specific inhibitor of nucleoside transport¹³, inhibits uptake

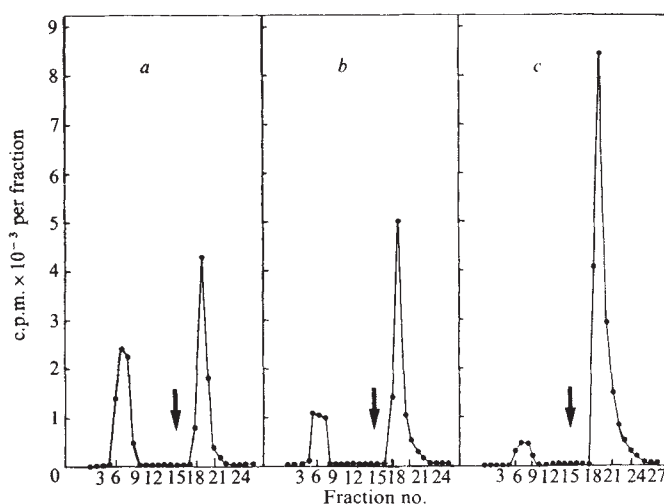


Fig. 3 Ion exchange chromatography of acid-soluble pools from quiescent cultures of 3T3 cells in different experimental conditions. Cultures of 3T3 cells were grown as described for Fig. 1 but in 90-mm diameter plastic dishes. The cultures were exposed for 30 min at 37 °C to DEM alone (*a*), DEM containing 0.5 nM NBMI (*b*) or DEM containing 10% foetal calf serum (*c*). At the end of the incubation the medium was removed rapidly, replaced by 3 ml of medium of the same composition in each case but containing 5-³H-uridine (28 Ci mmol⁻¹ 10 μ Ci ml⁻¹). After 6 s the radioactive medium was aspirated rapidly and the dishes were washed five times with 10 ml of ice-cold isotonic NaCl containing 100 nM NBMI. Then the cultures were extracted with 2 ml of 5% trichloroacetic acid for 1 h. The extracts from two cultures treated identically were combined and extracted five times with ether. (As control, we verified that 5-³H-uridine remains in the aqueous phase.) The aqueous phases were applied to columns (0.6 $\times$ 10 cm) of Bio-Rad AG1-x2 anion exchange resin (200-400 mesh) which had been converted to the formate form. Free uridine was eluted with 15 ml of H₂O; the phosphorylated nucleoside was subsequently eluted with 1 N HCl. The change to HCl is indicated by the arrows. Fractions of 1 ml were collected directly into scintillation vials; the samples 1 to 15 were adjusted to 1N HCl and then all the vials received 10 ml of Triton-toluene scintillation fluid. The radioactivity was determined in a Packard scintillation counter. To determine the amount of radioactive uridine that might remain in the extracellular space after washing, parallel cultures in DEM were exposed to 100 nM NBMI for 30 min, labelled for 3 s in the presence of the inhibitor to prevent the entry into the cells of any uridine and washed as described for the other cultures. In these conditions only a small amount of radioactivity was eluted in the tubes 5, 6, 7 and 8 (20, 66, 71 and 21 c.p.m. above background respectively) and thus, the radioactivity shown in the graphs represents only intracellular pools. The extensive washing procedure before extraction of the intracellular acid-soluble pools eliminates most of the extracellular uridine and the presence of NBMI during the washing prevents the efflux of free uridine¹⁴.

of uridine by intact 3T3 cells (Fig. 2a), but it does not alter nucleoside phosphorylation by cell-free homogenates prepared from parallel cultures (Fig. 2b). Thus, transport of nucleosides can be manipulated separately from their internal phosphorylation in 3T3 cells. When transport is made rate-limiting by addition of NBMI to quiescent cultures of 3T3 cells, the biphasic curve of uridine uptake is replaced by a straight line (Fig. 2c). When added to serum-stimulated cells, NBMI decreased the slope of the uptake curve (results not shown).

If phosphorylation is the rate-limiting step in the uptake of nucleosides by quiescent cells, more of the intracellular pool of uridine should be phosphorylated in serum-stimulated cultures than in the control ones, after a short exposure to labelled nucleoside. The phosphorylated nucleosides would be trapped inside the cell. We tested this possibility by measuring the radioactivity associated with the cells after labelling the cultures with ^3H -uridine for 5 s, rapidly replacing the radioactive medium with non-radioactive medium containing 1 mM uridine and incubating for another 10 s to allow for efflux of free ^3H -uridine. We found that 67.4% of the radioactivity remained associated with the cells in serum-treated cultures while 45.9% remained so associated in the non-stimulated cultures, a difference consistent with the prediction. To substantiate directly this conclusion, acid-soluble pools were labelled with high-specific activity ^3H -uridine for 6 s and were then analysed chromatographically. That intracellular phosphorylation rather than transport is rate-limiting in nucleoside uptake by non-stimulated cultures (Fig. 3a) is demonstrated by the fact that NBMI added at a low concentration (0.5 nM, which inhibits total uptake only by 25%) selectively reduces the labelling of the free-nucleoside pool by about 50% but does not modify the amount of radioactivity measured in the uridine-phosphate pool (Fig. 3b). If transport were rate-limiting, the inhibition of labelling by NBMI should be equal in both pools (free and phosphorylated uridine). Addition of serum to quiescent cultures changes considerably the composition of the acid-soluble pools (Fig. 3c); serum increases the radioactivity recovered in the nucleoside phosphate pool and considerably reduces that corresponding to the pool of cellular free uridine (Fig. 3c). Clearly, serum stimulates the rate of conversion of ^3H -uridine to phosphorylated nucleosides. Similar results were obtained in four independent experiments. The percentages of radioactivity found in the phosphorylated pools were $48 \pm 5\%$ and $88 \pm 4\%$ in the control and serum-activated cultures respectively, which are consistent with the washout experiments described here.

Taken together, our experiments support the notion that the stimulation of uridine uptake by serum in density-inhibited 3T3 cells results from an increase in the rate of internal phosphorylation rather than that of transport. How external growth-promoting factors regulate this rapid internal process warrants further investigation.

We thank Dr P. Plagemann for sending a copy of his manuscript before publication, Dr Z. I. Cabantchik for NBMI, Margaret Grimaldi and Ann Duffy for preparation of cell cultures and Drs J. Schneider and L. Heppel for helpful comments. W.D.S. thanks the NCI for financial support.

ENRIQUE ROZENGURT
WILFRED D. STEIN*
NOEL M. WIGGLESWORTH

Imperial Cancer Research Fund Laboratories,
Lincoln's Inn Fields,
London WC2, UK

Received 26 January; accepted 15 April 1977.

*Present address: Institute of Life Sciences, Hebrew University, Jerusalem, Israel.

- 1 Smith, J. A. & Martin, L. *Proc. natn. Acad. Sci. U.S.A.* **70**, 1263-1267.
- 2 Todaro, G. J., Matsuija, Y., Bloom, S., Robbins, A. & Green, H. in *Growth Regulating Substances for Animal Cells in Culture* (ed. by Defendi, A. & Stoker, M. G. P.) 87-98 (Wistar Institute Press, Philadelphia, 1967).
- 3 Cunningham, D. D. & Pardee, A. B. *Proc. natn. Acad. Sci. U.S.A.* **64**, 1049-1056 (1969).
- 4 Rozengurt, E. & Jimenez de Asua, L. *Proc. natn. Acad. Sci. U.S.A.* **70**, 3609-3612 (1973).
- 5 Stein, W. D. & Rozengurt, E. *Biochim. biophys. Acta* **419**, 112-118 (1976).
- 6 Rozengurt, E. & Stein, W. D. *Biochim. biophys. Acta* **464**, 417-432 (1977).
- 7 Jimenez de Asua, L. & Rozengurt, E. *Nature* **251**, 624-626 (1974).
- 8 Wohlheuter, R. M., Marz, R., Graff, J. C. & Plagemann, P. G. W. *J. Cell Physiol.* **89**, 605-612 (1976).
- 9 Plagemann, P. G. W. & Richey, D. *Biochim. biophys. Acta* **344**, 263-305 (1974).
- 10 Kessel, D. & Shurin, S. B. *Biochim. biophys. Acta* **163**, 179-187 (1968).
- 11 Ho, W. H. D. *Cancer Res.* **33**, 2816-2820 (1973).
- 12 Meyers, M. B. & Kreis, W. *Arch. Biochem. Biophys.* **177**, 10-15 (1976).
- 13 Cass, C. E., Jandette, L. A. & Paterson, A. R. P. *Biochim. biophys. Acta* **345**, 1-10 (1974).
- 14 Todaro, G. J. & Green, H. *J. Cell Biol.* **17**, 298-313 (1963).
- 15 Rozengurt, E. & Heppel, L. *Proc. natn. Acad. Sci. U.S.A.* **72**, 4492-4495 (1975).
- 16 Mizel, S. B. & Wilson, L. *Biochemistry* **11**, 2573-2578 (1972).

Activation of melanoma tyrosinase by a cyclic AMP-dependent protein kinase in a cell-free system

REGULATION of pigmentation is of considerable importance throughout the animal kingdom. Photoprotection, social behaviour, and a number of disorders like albinism, vitiligo, and melanoma are affected by the amount and distribution of melanin in the organism. Melanin is synthesised in melanosomes beginning with the oxidation of tyrosine to dihydroxy-phenylalanine (DOPA) by the enzyme tyrosinase. In Cloudman S91 melanoma cells, tyrosinase activity and melanisation are stimulated by elevated intracellular levels of cyclic AMP¹, apparently through the inactivation of an inhibitor of tyrosinase². We initiated a series of experiments to identify the molecules controlling tyrosinase activity. Kuo and Greengard's observations on the widespread occurrence of cyclic AMP-dependent protein kinases throughout the phyla suggested that protein phosphorylation reactions might be involved³. We describe here a cell-free system in which tyrosinase is activated following the addition of a cyclic AMP-dependent protein kinase isolated from melanoma cells. The kinetics of the reaction in the cell-free system are similar to those in intact cells. The activation of tyrosinase is completely dependent on the protein kinase and is enhanced by cyclic AMP, ATP, and magnesium. The activation involves the removal of an inhibitor of tyrosinase; in addition, a phosphoprotein phosphatase is present which is distinct from the inhibitor but which appears to antagonize the kinase-mediated reaction.

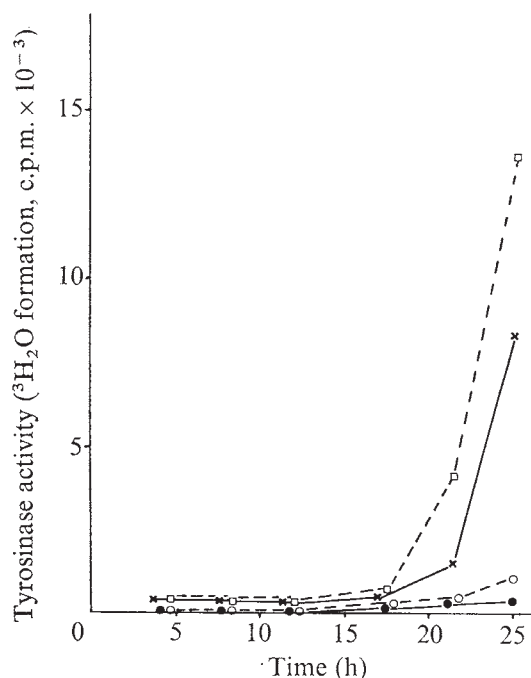
A cell line designated PS1-HGPRT-1 (ref. 4) was chosen for these studies because of its low basal tyrosinase activity which increases 5-50-fold about 15 h after the addition of melanocyte stimulating hormone (MSH) or dibutyryl cyclic

Table 1 Lack of effect of kinase on tyrosinase activity in extracts of cells exposed to MSH before collection

Time of incubation (h)	Additions to reaction	Tyrosinase activity (^3HOH production c.p.m.)
0	None	9,670
	Kinase	9,242
24	None	10,800
	Kinase	7,930

Cells were cultured for 5 d with MSH (2×10^{-7} M) present in the medium. They were collected and an S30 fraction was prepared as described in the legend to Fig. 1. Each reaction tube contained S30 (0.14 mg ml^{-1}), cyclic AMP (10^{-5} M), ATP (2×10^{-4} M), and magnesium acetate (1×10^{-3} M) in Tris-HCl (50 mM, pH 6.2). Protein kinase, when added, was at a concentration of 0.16 mg ml^{-1} . Reactions were incubated at 30°C , and at the times indicated 20 μl -samples were withdrawn and assayed for tyrosinase activity as in Fig. 1.

Fig. 1 Activation of tyrosinase in a cell-free system. Cells were cultured in monolayer as described previously⁶. Log-phase cultures were fed fresh medium within 24 h of collection. They were collected with 5 mM EDTA in Ca-Mg free Tyrodes salt solution at room temperature, collected by centrifugation (2,500g for 5 min), and washed three times in NaCl (156 mM, room temperature) by successive centrifugations. The washed pellets of cells were resuspended in 10 volumes of ice cold Tris-HCl (25 mM, pH 7.4) containing 0.5% Triton X-100, and lysed by mixing gently with a Vortex Genie mixer every 2–3 min. over a 20-min period. The lysed cells were centrifuged (30,000g, 15 min, 4 °C), the pellets were discarded, and the supernatant solution (S30) was dialysed against 2,000 volumes of Tris-HCl (25 mM, pH 7.4) for 5 h at 4 °C. The dialysed S30 fraction was then stored at –90 °C in convenient aliquots where it remained stable for at least 6 months. The S30 fraction contained 0.8 mg ml⁻¹ protein as determined by the method of Lowry *et al.*⁷. Cyclic AMP-dependent protein kinase (also referred to as kinase) was partially purified from tumours originating from the same cell line inoculated into DBA 2J mice. Tumours were removed from mice, rinsed with NaCl (156 mM), and homogenised in 3 volumes ice-cold KPO₄ (5 mM, pH 6.8). All further steps were at 4 °C. The homogenate was centrifuged first at 3,000g for 5 min to remove large particulate matter, and then at 30,000g for 15 min. An equal volume of hydroxyapatite (0.5 gml⁻¹ in 5 mM NaPO₄, pH 6.2) was added to the 30,000g supernatant fraction, the mixture was stirred (5 h), and then centrifuged (30,000g, 15 min). More than 90% of the cyclic AMP-dependent protein kinase activity was associated with the hydroxyapatite while all tyrosinase activity remained in the supernatant fraction. Kinase was eluted by suspending the hydroxyapatite in three volumes KPO₄ (0.3M, pH 6.8, containing mercaptoethanol, 4 mM, and glycerol, 10%), stirring (15 h, 4 °C), and then centrifuging (30,000g, 15 min 4 °C). The supernatant fraction contained the kinase activity and was dialysed against 1,000 volumes buffer A (KPO₄, 10 mM, pH 6.8, mercaptoethanol, 2 mM, glycerol, 10%) overnight at 4 °C and then placed on a DEAE-cellulose column equilibrated with the same buffer. Kinase was eluted from the column with a salt gradient from buffer A alone to buffer A containing KPO₄ (0.3 M, pH 6.8). Two prominent peaks of kinase were eluted from the column, peak I containing more than 80% of the kinase activity and peak II containing the remainder. This kinase elution profile was essentially the same as that seen in other tissues by several groups (for example, see ref. 8). Fractions containing peak I kinase were pooled and made 60% with (NH₄)₂SO₄. The kinase precipitated in these conditions and was stored in aliquots at 4 °C where it remained stable for at least 8 months. Although this procedure resulted in a substantial purification of the kinase, the preparation contained 10–15 protein bands when analysed by SDS-acrylamide gel electrophoresis. The preparation was free of tyrosinase activity, tyrosinase inhibitors, and protein phosphatase. The cell system contained the following components: S30 fraction (0.16 mg ml⁻¹). Tris-HCl (50 mM, pH 6.2), gentomycin (0.05 mg ml⁻¹), and where noted kinase (0.14 mg ml⁻¹), cyclic AMP (10⁻⁵ M), ATP (2 × 10⁻⁴ M), and magnesium acetate (10⁻² M). At times indicated samples were withdrawn and assayed for tyrosinase activity. The tyrosinase assay was a modification of the method of Pomerantz⁵. Samples (20 µl) were mixed with 10 µl of a solution containing ³H-tyrosine (50 µCi ml⁻¹, 1.5 × 10⁻⁴ M), dihydroxyphenylalanine (dopa, 2.5 × 10⁻⁴ M) and NaPO₄ (5 × 10⁻² M, pH 6.8). The mixture was incubated at 37 °C for 20 min, and 1 ml of activated charcoal (100 mg ml⁻¹ in citric acid, 0.1 M) was added. This mixture was then mixed briefly with a Vortex Genie mixer, centrifuged (2,000g, 5 min) and the supernatant fraction containing ³HOH was poured over a Dowex 50 column (1 cm in a Pasteur pipette) equilibrated with 0.1 M citric acid. The column eluate was counted in a liquid scintillation counter. Tyrosinase activity was linear over the 20 min incubation at 37 °C and was enhanced about fivefold by the addition of dopa. Control (●), added cyclic AMP, ATP, Mg²⁺ (○), added kinase (×), added kinase and cyclic AMP, ATP, Mg²⁺ (□). These experiments were repeated more than 10 times with similar results each time.



The supernatant fraction contained the kinase activity and was dialysed against 1,000 volumes buffer A (KPO₄, 10 mM, pH 6.8, mercaptoethanol, 2 mM, glycerol, 10%) overnight at 4 °C and then placed on a DEAE-cellulose column equilibrated with the same buffer. Kinase was eluted from the column with a salt gradient from buffer A alone to buffer A containing KPO₄ (0.3 M, pH 6.8). Two prominent peaks of kinase were eluted from the column, peak I containing more than 80% of the kinase activity and peak II containing the remainder. This kinase elution profile was essentially the same as that seen in other tissues by several groups (for example, see ref. 8). Fractions containing peak I kinase were pooled and made 60% with (NH₄)₂SO₄. The kinase precipitated in these conditions and was stored in aliquots at 4 °C where it remained stable for at least 8 months. Although this procedure resulted in a substantial purification of the kinase, the preparation contained 10–15 protein bands when analysed by SDS-acrylamide gel electrophoresis. The preparation was free of tyrosinase activity, tyrosinase inhibitors, and protein phosphatase. The cell system contained the following components: S30 fraction (0.16 mg ml⁻¹). Tris-HCl (50 mM, pH 6.2), gentomycin (0.05 mg ml⁻¹), and where noted kinase (0.14 mg ml⁻¹), cyclic AMP (10⁻⁵ M), ATP (2 × 10⁻⁴ M), and magnesium acetate (10⁻² M). At times indicated samples were withdrawn and assayed for tyrosinase activity. The tyrosinase assay was a modification of the method of Pomerantz⁵. Samples (20 µl) were mixed with 10 µl of a solution containing ³H-tyrosine (50 µCi ml⁻¹, 1.5 × 10⁻⁴ M), dihydroxyphenylalanine (dopa, 2.5 × 10⁻⁴ M) and NaPO₄ (5 × 10⁻² M, pH 6.8). The mixture was incubated at 37 °C for 20 min, and 1 ml of activated charcoal (100 mg ml⁻¹ in citric acid, 0.1 M) was added. This mixture was then mixed briefly with a Vortex Genie mixer, centrifuged (2,000g, 5 min) and the supernatant fraction containing ³HOH was poured over a Dowex 50 column (1 cm in a Pasteur pipette) equilibrated with 0.1 M citric acid. The column eluate was counted in a liquid scintillation counter. Tyrosinase activity was linear over the 20 min incubation at 37 °C and was enhanced about fivefold by the addition of dopa. Control (●), added cyclic AMP, ATP, Mg²⁺ (○), added kinase (×), added kinase and cyclic AMP, ATP, Mg²⁺ (□). These experiments were repeated more than 10 times with similar results each time.

AMP (Bt₂cAMP) to the culture medium. A 30,000g supernatant (S30) fraction of these cells was prepared and incubated with or without the additions of cyclic AMP-dependent protein kinase, cyclic AMP, ATP, and magnesium. Tyrosinase activity increased dramatically after a lag period of several hours (Fig. 1). The increase was enhanced by cyclic AMP, ATP, and magnesium, all of which were necessary for maximum response. The optimal pH for the reaction was 6.2 and the optimal temperature was 30 °C. The kinetics of activation were similar to those observed in intact cells exposed to Bt₂cAMP (Fig. 2). Cyclic AMP-dependent protein kinase activity was present in the S30 fraction, and if the concentration of the S30 was increased in the reaction mix, the activation reaction was no longer dependent on exogenously added kinase (data not shown). We first noticed the activation of tyrosinase when we inadvertently left a concentrated S30 fraction at room temperature and found the next day that the tyrosinase activity had increased by more than 100-fold.

When an S30 fraction was prepared from cells that had been exposed in culture to MSH for 5 d before collecting, the tyrosinase activity was high in fresh extracts and no activation occurred following the addition of protein kinase (Table 1). In these experiments the addition of kinase actually caused a slight decrease in tyrosinase activity.

To gain additional evidence for the role of protein kinase in the activation of tyrosinase we investigated the effects

Table 2 Effects of kinase modulator on protein kinase and the activation of tyrosinase

Experiment	Additions	Protein kinase activity (ATP ³² incorporation, c.p.m.)	Tyrosinase activity (³ HOH production, c.p.m.)
a	Kinase only	1,100	—
b	Kinase + modulator	320	—
c	Cell-free system	—	7,400
d	Cell-free system + modulator added at zero time	—	750
e	Cell-free system + modulator added at 24 h	—	7,650

Protein kinase modulator was prepared from calf brain through step 2 of the procedure of Donnelly *et al.*⁹. Experiments a and b were measurements of cyclic AMP-dependent protein kinase activity. Each reaction mix contained NaPO₄ (50 mM, pH 6.8), kinase (0.14 mg ml⁻¹), ATP³² (10⁻⁴ M, 0.2 Ci mm⁻¹), cyclic AMP (10⁻⁵ M), magnesium acetate (10⁻² M), and histones as substrate (Sigma, calf thymus type 11A, 200 µg ml⁻¹). Mixes (100 µl) were incubated for 5 min at 30 °C and the reactions were stopped by the addition of ice cold trichloroacetic acid (TCA 10%). Precipitates were washed several times in TCA and counted in a liquid scintillation counter. Experiments c–e were measurements of tyrosinase activity after 24 h in the cell-free system described in Fig. 1. Each reaction contained S30 fraction, kinase, cyclic AMP, ATP, and Mg²⁺. Modulator was added at a concentration of 0.34 mg ml⁻¹.

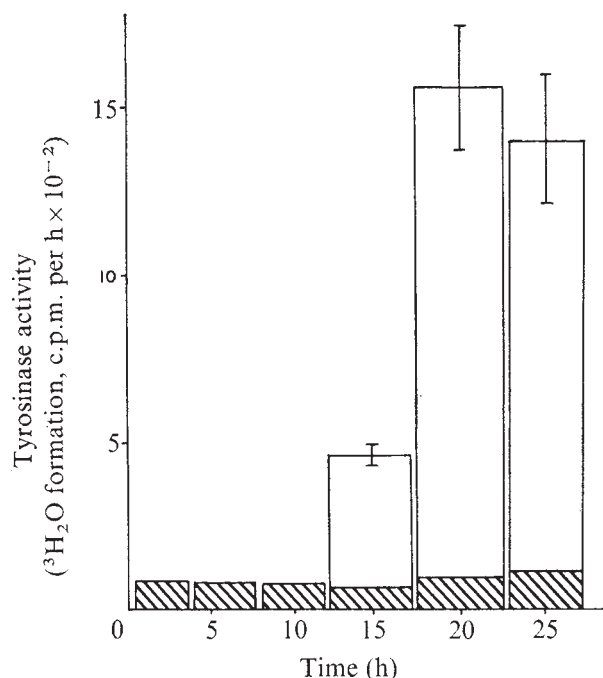


Fig. 2 Activation of tyrosinase in cultured melanoma cells by Bt_2cAMP . Cells (10^6) were cultured in F10 medium (4 ml) containing $3,5\text{-}^3H$ -tyrosine (New England Nuclear, $1\text{ }\mu\text{Ci ml}^{-1}$, final specific activity 88 Ci mol^{-1}) and at the times indicated 0.5-ml aliquots of medium were withdrawn and mixed with 1 ml charcoal as described in Fig. 1 to measure 3HOH Counts represent the amount of 3HOH formed during each time period. This experiment was carried out in parallel to that presented in Fig. 1. Bt_2cAMP (10^{-3} M) (open bar); control (cross hatched bar).

of an inhibitor of cyclic AMP-dependent protein kinases (protein kinase modulator)⁹. Kinase modulator inhibited both the protein kinase and the activation of tyrosinase but had no effect on activated tyrosinase (Table 2).

The kinase-mediated activation of tyrosinase involved the removal of an inhibitor of tyrosinase. Freshly-thawed S30 fractions contained tyrosinase inhibitory activity which disappeared after the activation of tyrosinase. The inhibitor

Fig. 3 Decay of phosphatase activity at 30°C . An S30 fraction was incubated at 30°C and at the times indicated aliquots ($20\text{ }\mu\text{l}$) were withdrawn, mixed with ^{32}P -labelled proteins described in the legend to Table 4, and phosphatase activity was measured.

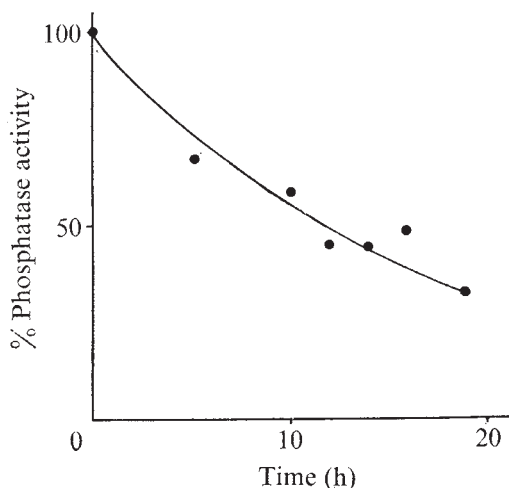


Table 3 Evidence for tyrosinase inhibitory activity in cell extracts*

Additions	Tyrosinase activity (c.p.m. 3HOH production)
Buffer only (controls)	22,422
a, freshly-thawed S30 fraction	7,500
b, S30 fraction incubated 55°C , 30 min	6,400
c, S30 fraction incubated 30°C , 24 h	1,400

*A cell-free system containing S30 fraction, kinase, cyclic AMP, ATP, and Mg^{2+} was incubated 24 h at 30°C as described in Fig. 1. At this time aliquots ($10\text{ }\mu\text{l}$) were withdrawn and mixed with buffer only in controls or additional S30 fraction ($10\text{ }\mu\text{l}$, 0.04 mg) which had been either a, freshly thawed; b, incubated at 55°C for 30 min, or c, incubated at 30°C for 24 h. 3H -tyrosine and dopa were added as in Fig. 1, the mixtures were incubated for 20 min at 37°C and the amount of 3HOH produced measured.

activity was stable to heating at 55°C for 30 min, or 30°C for 24 h (Table 3).

Phosphoprotein phosphatase (referred to as phosphatase) activity was present in freshly-thawed S30 preparations (Table 4). The disappearance of acid precipitable ^{32}P counts was not due to proteolytic digestion of the ^{32}P -labelled phosphoproteins since 3H -leucine-labelled proteins mixed into the assay tubes were not degraded during the same time period. Phosphatase activity in the S30 fractions was destroyed by heating the fractions at 55°C for 30 min. (Table 4) and had a half life of 11 h at 30°C (Fig. 3). The kinase-mediated activation of tyrosinase took place after the phosphatase had decayed substantially (cf Fig. 1). This finding raised the possibility that the lag period in the activation of tyrosinase was due to an antagonism between kinase-mediated phosphorylations and phosphatase-mediated dephosphorylations. Evidence supporting this possibility comes from the finding that alkaline phosphatase inhibited the activation reaction (Table 5). Since the phosphatase activity was heat-sensitive and tyrosinase inhibitory activity was not (Table 3), it is clear that the two are distinct from one another.

Data from several experiments show that the control of melanisation in Cloudman S91 melanoma cells is complex (see ref. 1 for a review). MSH causes an increase in intracellular levels of cyclic AMP by binding to cell surface receptors in the G2 phase of the cell cycle and activating adenylate cyclase¹⁰. Results from the cell-free system described here indicate that elevated levels of cyclic AMP activate a protein kinase which in turn causes an activation

Table 4 Phosphoprotein phosphatase activity in cell extracts

Time of Incubation (min)	Acid precipitable ^{32}P -proteins (c.p.m.)	Acid precipitable 3H -proteins (c.p.m.)
0	465	11,500
60	219	12,200
60, with heated S30	455	11,200

Substrate for the phosphoprotein phosphatase assay was prepared as follows: Freshly-thawed S30 was heated (55°C , 30 min) to destroy endogenous phosphatase activity. A mixture was then prepared containing the heated S30 (0.16 mg ml^{-1}) protein kinase (0.14 mg ml^{-1}), ATP³² (10^{-3} M), magnesium acetate (10 mM), NaPO_4 (50 mM , pH 6.8) in a total volume of 5 ml . The mixture was incubated at 30°C for 5 min, chilled on ice, and the ^{32}P -labelled proteins were separated from low molecular weight components on a Sephadex G25 column equilibrated with Tris-HCl (0.1 M , pH 7.4). Phosphatase activity was measured by adding the ^{32}P -labelled proteins to freshly thawed S30, incubating at 37°C , and following the disappearance of acid precipitable ^{32}P counts on filter disks with time. To distinguish phosphatase activity from protease activity, 3H -leucine-labelled proteins isolated from cells cultured in 3H -leucine were mixed into the reaction tubes.

of tyrosinase. These findings suggest a simple model in which the kinase catalyses the phosphorylation of a tyrosinase inhibitor and thus inactivates that inhibitor. Proof for such a model can come only when the individual components of the system have been purified. Data were presented previously indicating that intact cells contain a tyrosinase inhibitor which is inactivated on exposure of the cells to MSH (ref. 2).

The following observations lead us to conclude that the events we have observed in the cell-free system are the same as those which occur in cultured cells. (1) Tyrosinase inhibitory activity is removed in both cases. (2) Extracts from cells pretreated with MSH have high tyrosinase activity and cannot be activated further in a cell-free system. And (3), the kinetics of activation are similar. It

Table 5 Effects of alkaline phosphatase on the activation of tyrosinase

	Tyrosinase activity (c.p.m. ^3HOH production)
Cell-free system	12,700
Cell-free system + alkaline phosphatase	3,500

A cell-free system containing S30 fraction, kinase, cyclic AMP, ATP, and Mg^{2+} was incubated for 24 h in the presence or absence of alkaline phosphatase (Boehringer Mannheim, calf intestine, 0.05 mg ml^{-1}).

should be noted that this report describes only the activation of tyrosinase, that is, the enzyme which catalyses the conversion of tyrosine to dopa. In none of the experiments did we see an increase in melanin formation, a result which we do not understand. In the melanin biosynthetic pathway, tyrosinase catalyses the oxidation of tyrosine to dopa and then of dopa to dopa quinone. Since the results reported here deal only with the first step in the reaction, it is possible that the second step is under separate controls and was not activated in our experiments. Using the cell-free system, we hope eventually to demonstrate increased melanisation and to identify the molecules controlling tyrosinase activity.

We thank Marilyn Sansone and John Hendee for technical assistance, and Dr Aaron B. Lerner for encouragement and support. A.K. was the recipient of an NIH post-doctoral fellowship and carried out part of this work in the laboratory of Dr David Seligson. Supported by grants from the NIH, American Cancer Society, and the Swebilius Foundation.

ANN KÖRNER*
JOHN PAWELEK†

Departments of Dermatology and Cytology,
Yale University School of Medicine,
New Haven, Connecticut 06510

Received 7 February; accepted 12 April 1977.

*Present address: Department of Biology, Yale University, New Haven, Connecticut 06511

†To whom requests for reprints should be addressed.

- 1 Pawelek, J. M. *J. invest. Derm.* **66**, 201–209 (1976).
- 2 Wong, G. & Pawelek, J. *Nature* **255**, 644–646 (1975).
- 3 Kuo, J. F. & Greengard, P. *Proc. natn. Acad. Sci. U.S.A.* **64**, 1349–1355 (1969).
- 4 Pawelek, J., Halaban, R. & Christie, G. *Nature* **258**, 539–540 (1975).
- 5 Pomerantz, S. H. *Science* **164**, 838–839 (1969).
- 6 Wong, G. & Pawelek, J. *Nature* **241**, 213–215 (1973).
- 7 Lowry, O. H., Rosenbrough, A. L., Farr, A. L. & Randall, R. J. *J. biol. Chem.* **193**, 265–273 (1951).
- 8 Reimann, E. M., Walsh, D. A. & Krebs, E. G. *J. biol. Chem.* **246**, 1986–1995 (1971).
- 9 Donnelly, T. E., Kuo, J. F., Reyes, P. L., Liu, Y.-P. & Greengard, P. *J. biol. Chem.* **248**, 190–198 (1973).
- 10 Varga, J. M., DiPasquale, A., Pawelek, J., McGuire, J. S. & Lerner, A. B. *Proc. natn. Acad. Sci. U.S.A.* **71**, 1590–1593 (1974).

Properties of an identified histamine-containing neurone

THE cellular role of neuronal histamine is still not understood, although a transmitter function is suspected¹. Advances have been restricted largely because of the lack of experimentally amenable, peripheral, histamine-containing systems in the vertebrates. As an alternative approach to the problem, we have sought an identifiable histamine-containing neurone in the central ganglia of a molluscan species. Previous studies on repeatedly identifiable neurones of this group have facilitated study of individual neurones known to contain, for instance, dopamine or 5-hydroxytryptamine, and the synaptic connections made by these neurones². Here we briefly describe the localisation of one particular histamine-containing neurone, its content of histamine, ability to take up histamine from an incubation medium, the type of granulated vesicles it contains and the influence of certain transmitter substances on its electrical activity.

Experiments were made on the pond snail, *Lymnaea stagnalis*. Neurones were initially screened using light microscope autoradiography after exposure to tritiated histamine, on the assumption that those neurones which contain histamine would also preferentially take up the amine from the extracellular fluid. The central ganglia were incubated at 18–20 °C in 100 μl of physiological solution³ containing 25 μM 2,5- ^3H -histamine dihydrochloride (1 Ci mmol^{-1} , Radiochemical Centre, Amersham) for 2 h. After washing in a continuous saline flow for 30 min, the tissues were fixed in 1% glutaraldehyde in 0.067 M phosphate buffer (pH 7.4), dehydrated through ethanol series and embedded in paraffin wax. Serial sections 7 μm thick, were coated with Kodak AR 10 stripping film, exposed for 10–14 d in the dark and developed in Kodak D 19. A specific population of neurones was found to be repeatedly labelled after this treatment. All of these neurones were small (<50 μm diameter) and not readily identifiable in intact preparations, with the exception of a single large neurone (Fig. 1) situated on the anterior dorsal surface of the visceral ganglion. The perikaryon of this neurone is 90–170 μm across its major axis and can be readily located in the majority of preparations by virtue of its size and position.

Histamine was assayed in extracts of individually dissected perikarya using a radioenzymatic method⁴. The

Fig. 1 Autoradiogram of a section of the visceral and left parietal ganglia of *Lymnaea* after incubation in physiological solution containing ^3H -histamine. Dense aggregations of silver grains are seen over the perikaryon of the giant visceral neurone (large arrow), and over portions of one of its main axon branches (small arrows) in the neighbouring left parietal ganglion. Scale bar represents 300 μm .



Table 1 Intracellular histamine concentration

Neurone	Intracellular histamine concentration $\pm$ s.e.m. (μ M)
Giant visceral neurone (<i>Lymnaea</i>)	275 ± 11.7 ($n = 4$)
Dopamine-containing neurone (<i>Planorbis</i>)	38.7 ± 3.8 ($n = 3$)
Non-aminergic neurone (<i>Lymnaea</i>)	None detectable ($n = 3$)

large neurone described above which took up histamine was found to contain a high level of histamine, whereas other identified neurones, a non-amine-containing neurone of *Lymnaea* (this neurone, located in a similar position to the VDI of Winlow and Benjamin⁵, reacted negatively when the ganglia were examined by formaldehyde histo-fluorescence for primary amines) and the giant dopamine neurone of *Planorbis*⁶, contained much less histamine (Table 1). High specific histidine decarboxylase activity is associated with the part of the visceral ganglion containing the perikaryon of the histamine-rich neurone and recent experiments show that there is an enzyme within the perikaryon capable of synthesising histamine from histidine (J.D.T., unpublished).

For electron microscopy, individual neurones were dissected free from glutaraldehyde-fixed ganglia, post-fixed in 1% osmium tetroxide in 0.2 M veronal buffer (pH 7.4), stained in 1% uranyl acetate, dehydrated through acetone series and embedded in Epon. Examination of thin sections of the identified histamine-neurone by electron microscopy (Philips EM 301) revealed large aggregations of membrane bound dense-cored vesicles, of 80–200 nm diameter (Fig. 2), and extensive and highly organised rough endoplasmic reticulum in the cell cytoplasm, as well as numerous elongated mitochondria and lysosome-like bodies. The dense cores are larger than those of vesicles reported in the perikarya of molluscan dopamine-containing⁶ and 5-hydroxytryptamine-containing⁷ neurones. The vesicles also seem to occur in much larger aggregations than in these other amine-containing neurones.

The histamine neurone was readily located in the isolated ganglionic preparation for intracellular electrical

recordings using conventional techniques. Small excitatory postsynaptic potentials and inhibitory postsynaptic potentials were recorded from the neurone in different preparations, without applying any stimulation. Occasionally the excitatory input was sufficient to cause the cell to fire action potentials. The effects of some transmitter substances were tested on the activity of the neurone. These compounds were applied iontophoretically from fine-tipped micropipettes placed close to the perikaryon, using current pulses of 50–300 nA for durations of 0.5–2.5 s. Acetylcholine caused a biphasic depolarising–hyperpolarising response. The first phase of the response was comparatively fast and blocked with 10^{-4} M hexamethonium bromide. The second phase was not blocked with this concentration of hexamethonium; it was very rapidly desensitised and chloride dependent. 5-Hydroxytryptamine also produced a rapidly desensitising, chloride-dependent hyperpolarising response. In contrast, iontophoretically applied histamine, dopamine, glutamate and γ -aminobutyric acid were without any obvious effects. But addition of histamine acid phosphate directly to the perfusion fluid resulted in a gradual depolarisation of the neurone, due to an increase in membrane conductance, and spike firing.

The giant visceral neurone has numerous axon branches with ramifications in other ganglia. Some branches leave the central ganglia for the periphery (J.D.T., S. Logan and G.A.C., unpublished data). As yet, however, the functional role of the neurone is not known.

In summary, there is a giant histamine-containing neurone in the visceral ganglion of *Lymnaea stagnalis*. The histamine concentration in this neurone (275 μ M) is comparable with that recently reported for the C2 neurones (300–400 μ M) located in the cerebral ganglia of *Aplysia californica*⁸. The *Lymnaea* histamine-neurone contains large aggregations of granulated vesicles of distinctive appearance and it is capable of taking up histamine from the surrounding medium. The neurone can be readily located for electrophysiological experiments and would seem to provide a suitable model system for analysing the cellular role of neuronal histamine.

We thank Professor J. C. Schwartz for assistance in histamine and histidine decarboxylase estimation. This work was supported by EMBO. J.D.T. was a Wellcome Research Scholar during the major part of this project.

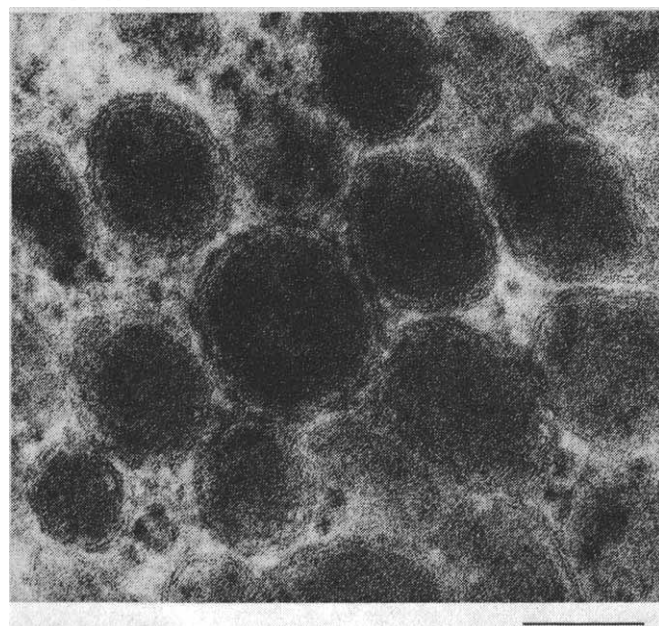
J. D. TURNER
G. A. COTTRELL

Wellcome Laboratories of Pharmacology,
Gatty Marine Laboratory,
University of St Andrews,
Fife, UK

Received 14 March; accepted 19 April 1977.

- Schwartz, J. C. *Life Sci.* **17**, 503–518 (1975).
- Cottrell, G. A. *Neuroscience* **2**, 1–18 (1977).
- De Vlieger, T. A. *Neth. J. Zool.* **18**, 205–154 (1968).
- Taylor, K. M. & Snyder, S. H. *J. Neurochem.* **19**, 1343–1358 (1972).
- Winlow, W. & Benjamin, P. R. in *Neurobiology of Invertebrates, Gastropoda Brain Tihany 1975* (ed. Salanki, J.) 41–59 (1976).
- Pentreath, V. W., Berry, M. S. & Cottrell, G. A. *Cell Tiss. Res.* **151**, 369–384 (1974).
- Pentreath, V. W., Osborne, N. N. & Cottrell, G. A. *Z. Zellforsch.* **143**, 1–20 (1973).
- Weinreich, D. in *Neurobiology of Invertebrates, Gastropoda Brain, Tihany 1975* (ed. Salanki, J.) 239–251 (1976).

Fig. 2 High magnification electron micrograph of the vesicular inclusions in the perikaryon of an isolated giant *Lymnaea* visceral neurone. The vesicles (80–200 nm diameter) have limiting membranes surrounding large electron-dense cores and are found in large aggregations in the cytoplasm. Scale bar represents 0.1 μ m.



5-Hydroxytryptamine receptors on visceral primary afferent neurones in the nodose ganglion of the rabbit

5-HYDROXYTRYPTAMINE (5-HT) is known to be a powerful activator of the sensory nerve endings¹ and a potent stimulant of sympathetic ganglia^{2–4}. Very little is known, however, about the nature of 5-HT receptors and the ionic basis of the 5-HT-induced membrane potential changes^{5,6}.

Here I report the existence of 5-HT receptors on the soma of afferent neurones of the nodose ganglion of the vagus and the ionic requirements of the response elicited by activation of these receptors.

The nodose ganglion was excised from the rabbits anaesthetised with pentobarbitone (30 mg per kg body weight intravenously) and intracellular recordings were made from the somata of neurones by methods essentially similar to that of Jaffe and Sampson⁷. 5-HT was applied either by superfusion as an addition to Krebs solution or by iontophoresis. Electrodes used for iontophoresis were filled with 0.1 M 5-HT adjusted⁸ to pH 3.8–4.0 and had tip resistances of 100–200 M Ω .

A majority of neurones (76 out of 88) were depolarised by 5-HT. Figure 1a shows a typical response to superfusion with a solution containing 5×10^{-5} M 5-HT. In most instances, the onset of the depolarisation was rapid and concentration dependent. The minimal effective concentration to produce the depolarisation was 10^{-7} M, and the maximal response was obtained at concentrations greater than 10^{-4} M. Repetitive firing frequently occurred during the rising phase of the depolarisation. The depolarisation was followed by an almost equally rapid repolarisation, then a hyperpolarisation (after-hyperpolarisation) of approximately 5 mV. The after-hyperpolarisation was in turn followed by a long-lasting depolarisation of a few millivolts.

Iontophoretic application of 5-HT elicited a response similar to that induced by superfusion. As seen in Fig. 1b, a 10^{-8} -A pulse (80 ms) sufficed to produce a depolarising response and a typical biphasic response was induced with a 10^{-7} -A pulse.

All the components of a 5-HT response were associated with a reduction of membrane resistance as by the decrease in the amplitude of the electrotonic potentials superimposed on the response (Fig. 1a).

In an attempt to determine the ionic species involved, the 5-HT responses were studied in various ionic media and at different membrane potentials. Replacement of external sodium by choline or Tris (hydroxymethyl)aminomethane abolished not only the rapid and residual depolarisations but also the after-hyperpolarisation. Removal of external potassium caused a slight decrease of the rapid depolarisation, a marked enhancement of the after-hyperpolarisation and no significant alteration in the residual depolarisation. Replacement of external chloride by acetate reduced the resting potential by approximately 15 mV and nearly halved the amplitude of 5-HT response. This reduction in amplitude was, however, eliminated when the membrane potential was restored by passing hyperpolarising current through the microelectrode.

When the membrane was depolarised stepwise, the rapid depolarising response was reduced accordingly and finally reversed at a certain level (-0.1 ± 2.0 mV s.e., $n=8$). This reversal level was at least 30 mV below the sodium equilibrium potential determined from the peak of the action potential. The after-hyperpolarisation could be reversed at -87 ± 2.5 mV ($n=8$) at which voltage the after-hyperpolarisation of the action potential also inverted. The reversal levels of the rapid depolarisation and the after-hyperpolarisation were unaltered by the replacement of external chloride with acetate.

These findings suggest that during the rapid initial depolarising response to 5-HT, there is a simultaneous increase in sodium and potassium conductance and during the after-hyperpolarisation only potassium conductance is increased. Since, however, both the initial depolarising and hyperpolarising responses are abolished in sodium-free media, the possibility arises that the after-hyperpolarisation is triggered by the entry of sodium ions. The fact that the residual depolarisation was abolished by deprivation of sodium but unaltered by withdrawal of potassium indicates

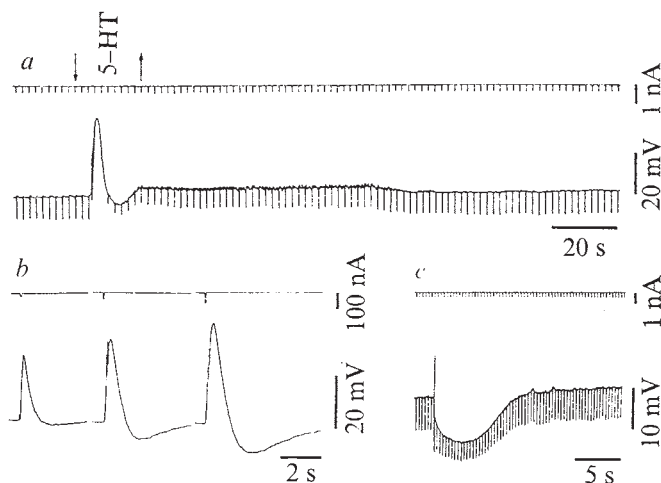


Fig. 1 5-HT-induced responses in afferent neurones of the rabbit nodose ganglion. Intracellular records from three different neurones show; *a*, depolarising response to 5×10^{-5} M 5-HT applied by superfusion for 20 s (between two arrows). Electrotonic potentials were elicited by square pulses (0.3 nA, 100 ms) every 2 s. *b*, Depolarising responses to 5-HT iontophoretically by current pulses (80 ms) of 20 nA (left), 35 nA (middle) and 85 nA (right). *c*, Hyperpolarising response to 5-HT iontophoretically by a current pulse of 80 nA and 100 ms. Electrotonic potentials were elicited by square pulses (0.25 nA, 50 ms) every 0.3 s. In each record the upper trace shows electrotonic (*a* and *c*) or iontophoretic (*b*) current pulses and the lower trace the membrane potential changes. Resting potentials of the three neurones were -58 mV (*a*), -62 mV (*b*) and -55 mV (*c*). Action potentials that appeared on the rising phase of 5-HT-induced depolarisation were attenuated by the ink-writing recorder.

that the residual component is associated with a small but sustained sodium permeability.

In a small number of neurones (5 of 88), 5-HT evoked a simple hyperpolarising response with no preceding or succeeding depolarisation as shown in Fig. 1c. The response varied in amplitude from 3.8 to 11.2 mV and in duration from 5.4 to 10.4 s and was consistently accompanied by a reduction of membrane resistance (Fig. 1c). Removal of potassium enhanced the hyperpolarisation, and displacement of membrane potential beyond -90 mV reversed its polarity. These characteristics reflect that an increased potassium conductance is the major, if not the exclusive, cause of this hyperpolarising response.

A similar group of neurones (7 of 88) did not show any response to 5-HT applied by iontophoresis and by superfusion.

Thus the majority of vagal afferent neurones are endowed with 5-HT receptors which are most commonly depolarising and only rarely hyperpolarising. The functional role of these receptors is obscure at the present. Presumably they could be a remnant of an embryonic stage in which 5-HT receptors migrate from the cell soma to the axon terminals. A number of authors have suggested that the depolarising response of the same population of neurones to GABA is similarly mediated by residual receptors (ref. 9). Much in the same way the 5-HT receptors would offer an opportunity to investigate their physiological and pharmacological characteristics in conditions which cannot as yet be achieved at the axon terminal.

This study was supported by the Ministry of Education, Science and Culture of Japan.

H. HIGASHI

Department of Physiology,
Kurume University School of Medicine,
Kurume, Japan

Received 22 February; accepted 5 April 1977.

¹ Paintal, A. S. *Pharmac. Rev.* **16**, 341–380 (1964).

² Haefely, W. in *Handbook of Experimental Pharmacology XXXIII* (eds Blashko, H. & Muscholl, E.) 661–725 (Springer, Berlin, Heidelberg & New York, 1972).

- ³ Haefely, W. *Naunyn-Schmiedeberg Arch. exp. Path. Pharmac.* **281**, 145–165 (1974).
⁴ North, R. A. & Wallis, D. I. *Br. J. Pharmac.* **59**, 505p (1977).
⁵ Watanabe, S. & Koketsu, K. *Experimenta* **29**, 1371–1372 (1973).
⁶ Wallis, D. I. & Woodward, B. *Br. J. Pharmac.* **55**, 199–212 (1975).
⁷ Jaffe, R. A. & Sampson, S. R. *J. Neurophysiol.* **39**, 802–815 (1976).
⁸ Jordan, L. M., Frederickson, R. C. A., Phillis, J. W. & Lake, N. *Brain Res.* **40**, 552–558 (1972).
⁹ Desheues, M., Feltz, P. & Lamour, Y. *Brain Res.* **118**, 486–493 (1976).

A regenerating neurone in the leech can form an electrical synapse on its severed axon segment

WHEN the nervous system develops, its constituent neurones establish connections with certain selected elements while rejecting others in a fashion that gives the completed system the precision it requires to function. Such specificity is also manifested during regeneration in the central nervous system (CNS)^{1–3}. For example, regeneration and recognition have been demonstrated with electrophysiological techniques in the leech CNS^{4,5}, where individual sensory neurones can select their normal postsynaptic targets from among apparently hundreds of alternatives, re-establishing chemical synaptic connections. The leech CNS has also provided an example of neurones re-establishing electrical continuity after surgical disruption of a connecting axon⁶. Such electrically connected 'S-cell' interneurons, one of which is situated in each of the 21 segmental ganglia of the leech, are linked at the ends of their axons by electrical synapses across which large molecules cannot pass⁷. The synapse between two S interneurons occurs in the axon bundles (connectives) that join ganglia. The discovery of this junction and the use of the enzyme horseradish peroxidase (HRP) as an intracellular marker have permitted a detailed morphological and physiological analysis of the steps by which a regenerating axon re-establishes synaptic contact with its proper target. We report here that one such step can be the formation of an electrical synapse between the regenerating neurone and its own severed axon segment, thereby restoring the neurone's functional integrity.

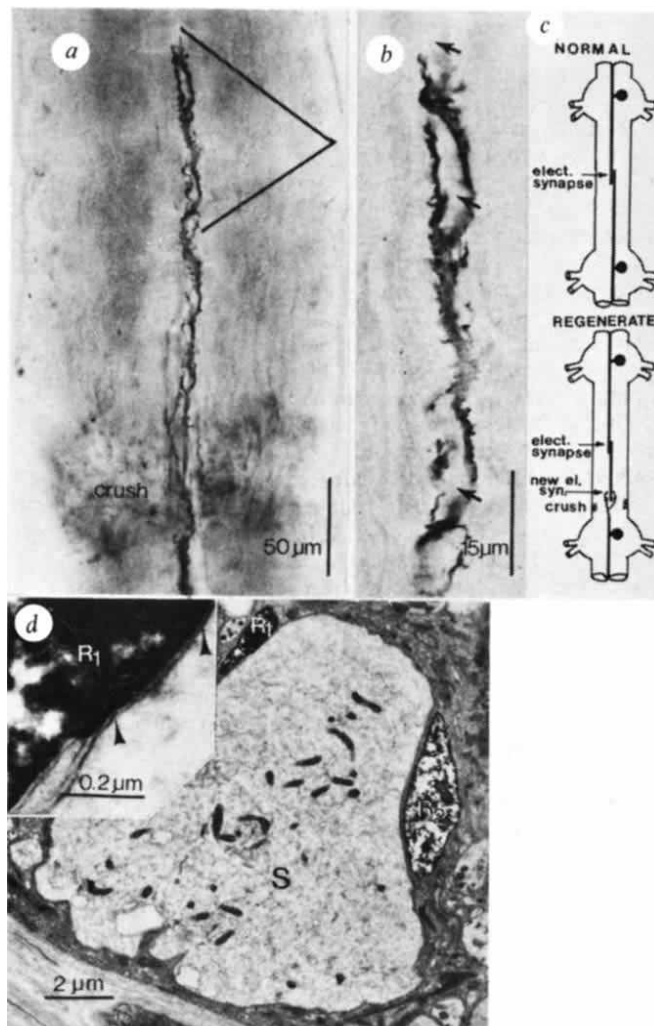
Except at the extreme ends of the ventral nerve cord, the axons of S cells meet at about the midpoint along the connectives between the ganglia (Fig. 1c). This single region of contact between neighbouring S cells is the site of the electrical synapse, across which nerve impulses can pass in either direction as they traverse the entire length of the intact nerve cord. In the present experiments the cord was crushed close to one ganglion (either 8, 9, 10 or 11) so that the electrical synapse between S cells was not directly affected by the lesion. This procedure severs all axons including those in the bundle of nearly a hundred in which the S-cell axon travels. Animals were allowed to recover in spring water at 16 °C, the usual holding temperature. Distal to the crush the operation leaves an isolated axon segment about 1 or 2 mm long that remains viable and linked through an electrical synapse to the adjacent S cell, termed here the 'target' cell of the regenerating process.

The extent of regeneration was determined by removing a short chain of ganglia including the lesion from the animal, and then recording from the chain in the leech Ringer's solution^{8,9}. Electrical coupling between cells was detected with intracellular microelectrodes inserted into S-cell somata and with suction electrodes applied to the connectives. Intracellular microelectrodes were filled either with 4 M potassium acetate or with a solution of 2% horseradish peroxidase in 0.2 M potassium chloride. To mark the entire arborisation of an S cell, peroxidase solution was injected under pressure into the S-cell soma through the recording microelectrode, the chain of ganglia incubated for 6–12 h in a modified L-15 culture medium^{10–11}, and the histochemical reaction and subsequent treatment for light or electron microscopic observation performed as previously

described^{12–13}. The diaminobenzidine reaction product is not seen in uninjected cells that are electrically coupled to the injected neurone, but owing to the activity of endogenous peroxidases some reaction product can appear in peroxisomes in all cell somata, especially in culture medium.

In six experiments the restoration of electrical coupling and subsequent conduction of impulses between S cells occurred in less than 4 weeks. In these preparations there was no anatomical contact between neighbouring S cells, as determined with the HRP marking technique. Rather, when HRP was injected into both the regenerating injured S cell and its target S cell (which showed no sign of growth) the reaction product revealed a gap as large as a millimeter between the stained axons (Fig. 1a). This distance decreased during the month following the lesion. In these instances

Fig. 1 The regenerating axonal processes of the S cell surround the neurone's severed distal segment. *a*, The axon proximal to the crush (below) branches as it crosses the crush in the centre of the connective and remains branched as it forms a basket of processes along the site of the distal segment. The marked cell ends abruptly several hundred micrometres before reaching the next S cell (not shown) whose extent had also been determined with peroxidase injections. The growing tip is shown in *b*. In this plane of focus the marked processes are seen to surround portions (indicated at three locations by arrows) of the distal segment (18 d). *c*, Schematic diagrams from peroxidase-injected preparations indicating the normal and experimentally induced connection (anterior at top). *d*, Electron micrograph of connective cross section showing two stained processes of the regenerating neurone (R_1 and R_2) near the distal segment (S), identified by its large size, dorsal position in Faivre's nerve and light cytoplasm. Inset: where the regenerating axon (R_1) apposes the segment are points at which the membranes are separated by no more than 4–5 nm (arrowheads), the presumed gap junctions (34 d).



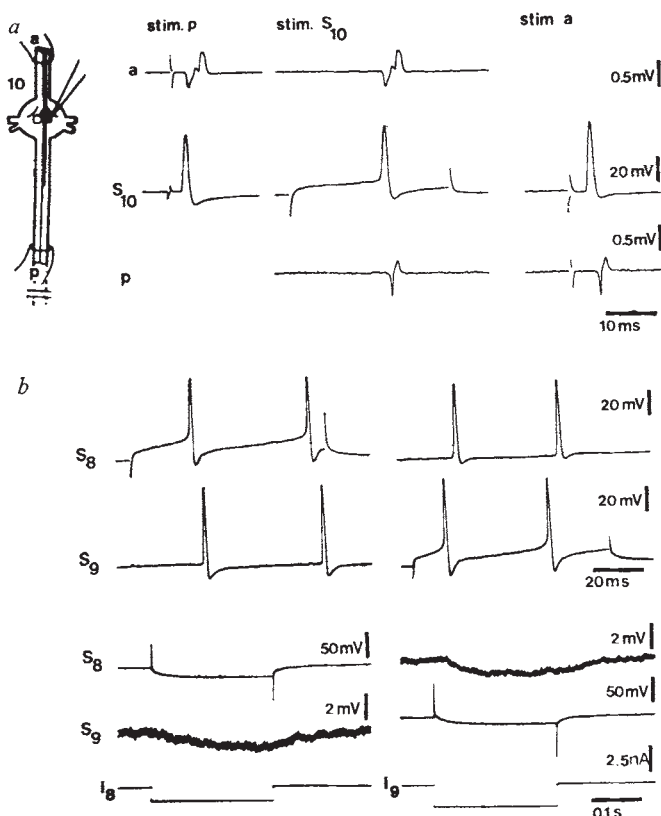


Fig. 2 a, The distal axon segment (here of S cell in ganglion 11) remains functionally connected to the adjacent target S cell (in ganglion 10) after crushing close to ganglion 11 (19 d previously). Suction electrodes record anteriorly (a) and posteriorly (p) to ganglion 10, in which the target S cell (S₁₀) has been penetrated with a recording microelectrode. Prior recording and stimulation posterior to the crush (=) demonstrated that the S-cell connection from ganglion 11 had not regenerated. The recordings here illustrate respectively that (stim. p) threshold stimulation at p excites S₁₀ whose impulse is also recorded at a; (stim. S₁₀) direct activation of S₁₀ with a depolarising pulse of current through the recording microelectrode activates not only its own axon recorded at (a), but also the axon of the distal stump recorded at p; and (stim. a) anterior connective stimulation at threshold directly activates S₁₀ and subsequently the distal stump at p. In the diagram on the left, the thickened line in the connectives represents the peroxidase-stained axon of S₁₀, which terminated more than one millimetre from point p. At this distance the suction electrode (p) would not have directly activated or recorded from S₁₀. **b**, Coupling of S-cells through the distal segment. The connective between ganglia 8 and 9 was crushed 20 d previously. S cells (S₈ and S₉) that, on subsequent marking with peroxidase, were found not to have regenerated a direct contact nevertheless show normal functioning. Upper: impulses generated in S₈ with the recording microelectrode are propagated to S₉ and vice versa. Lower: hyperpolarising currents injected into S₈ or S₉ spread into the adjacent S cell, indicating good coupling through the uninjected process.

of coupled neighbours not in direct contact, the regenerating neurone formed a basket of processes around a 5–8- μ m cylinder that in the electron microscope is identified as its own distal segment (Fig. 1b, c and d). Glial processes are seen between most of the regenerating and old axons, but at the sites of contact between the regenerating process and the distal segment are occasional points of close apposition. Tilting of the specimen in the electron microscope reveals that the membranes are separated by no more than 4–5 nm. These areas of contact, though small, resemble gap junctions between neighbouring S cells in the normal connective⁷ and those found in other invertebrates¹⁴. Electron microscopic examination of cross sections through the connective in the region of the discontinuity between injected S cells confirms that S-cell regeneration is not complete and that the distal axon segment is linking the S cells.

During the month after the lesion is made the distal axonal segment can retain its integrity and remain functionally connected to the target S cell through the old electrical junction. The S-cell axon normally has a low threshold and generates the largest impulses recorded from the connective. These physiological properties and nearly normal ultrastructure are retained by the distal axon segment during the first month. Figure 2a shows that before the two S cells have become functionally reconnected the isolated distal segment can still excite and be excited by the adjacent target S cell.

In two or three weeks the coupling between adjacent S cells through the isolated axonal link develops a nearly normal⁶ strength with impulses conducted in both directions (Fig. 2b). This coincides with an increasingly extensive association between the regenerating neurone and its distal segment, which itself remains connected to the target S cell.

When coupled neurones are examined at progressively later times, the distance shortens between the axon tips of the injected adjacent S cells. For example, the cell in Fig. 1d had grown 700 μ m to within 150 μ m of the target S cell. After about 1 month no separation between coupled cells is seen. This suggests that neurones already functionally connected through the distal segment can eventually re-establish direct contact at the normal site of synapse through continued regeneration of the lesioned neurone. Growth beyond the normal synaptic contact has not been observed even after six months.

Regenerating S cells establish strong electrical synapses specifically with S cells. In several experiments electrical coupling was not detected between the regenerating S cell in one ganglion and cells near the S cell in adjacent ganglia. Moreover, an impulse in the regenerating S cell will evoke only an S-cell impulse recorded in the connectives. Certainly the close anatomical association between the regenerating S cell and its own distal segment suggests a specific and exclusive recognition of cell type.

The electrical synapse formed by the regenerating neurone on its own distal segment is functionally indistinguishable from a direct fusion with the segment, except that large molecules such as HRP (molecular weight, 40,000) are unable to cross the junction. This raises the possibility that for other systems in which fusion has been invoked to explain rapid and complete regeneration (see refs 15–17 and compare ref. 18), an electrical synapse has also formed.

Knowledge of the exact point of synapse between neighbouring, electrically coupled S neurones in the leech has provided a tool for following a regenerating neurone as it re-establishes a fully functional and structurally intact electrical synapse. In some cases transmission was rapidly restored when the regenerating neurone formed first an electrical synapse with its own severed but still functioning distal axon segment. The segment seems to be an imperfect target for the regenerating neurone, however, for the neurone continues growing along the axon segment to the normal site of synapse, where growth ceases.

We thank Ms Barbara Thomas for technical assistance and Drs Joel Brown, Diana Card, Doug Fambrough, Eric Frank, Skip Hunt, Jan Jansen, Don Kennedy, Jack McMahan and John Nicholls for helpful suggestions. S.C. is supported in part by a U.S. NIH fellowship.

SALVATORE CARBONETTO
KENNETH J. MULLER

Carnegie Institution of Washington,
Department of Embryology,
115 West University Parkway,
Baltimore, Maryland 21210

Received 15 March; accepted 6 April 1977.

¹ Gaze, R. M. & Keating, M. J. *Nature* 237, 375–378 (1972).

- 2 Hunt, R. K. & Jacobson, M. *Curr. Tops. Devl Biol.* **8**, 203-259 (1974).
- 3 Yoon, M. *Cold Spring Harb. Symp. quant. Biol.* **40**, 503-519 (1976).
- 4 Baylor, D. A. & Nicholls, J. G. *Nature* **232**, 268-269 (1971).
- 5 Jansen, J. K. S. & Nicholls, J. G. *Proc. natn. Acad. Sci. U.S.A.* **69**, 636-639 (1972).
- 6 Frank, E., Jansen, J. K. S. & Rinovik, E. *J. comp. Neurol.* **159**, 1-13 (1975).
- 7 Muller, K. J., Carbonetto, S. T. & Thomas, B. *Carnegie Inst. Yrbk* **75**, 90-97 (1976).
- 8 Kuffler, S. W. & Potter, D. D. *J. Neurophysiol.* **27**, 290-320 (1964).
- 9 Nicholls, J. G. & Baylor, D. A. *J. Neurophysiol.* **31**, 740-756 (1968).
- 10 Miyazaki, S., Nicholls, J. G. & Wallace, B. G. *Cold Spring Harb. Symp. quant. Biol.* **40**, 483-493 (1976).
- 11 Miyazaki, S. & Nicholls, J. G. *Proc. R. Soc. B* **194**, 295-311 (1976).
- 12 Muller, K. J. & McMahan, U. J. *Anat. Rec.* **181**, 432 (1975).
- 13 Muller, K. J. & McMahan, U. J. *Proc. R. Soc. B* **194**, 481-499 (1976).
- 14 Perrachia, C. J. *Cell Biol.* **57**, 54-65 (1973); **57**, 66-76 (1973).
- 15 Hoy, R. R., Bittner, G. D. & Kennedy, D. *Science* **156**, 251-252 (1967).
- 16 Kennedy, D. & Bittner, G. D. *Cell Tiss. Res.* **148**, 97-110 (1974).
- 17 Birse, S. C. & Bittner, G. D. *Brain Res.* **113**, 575-581 (1976).
- 18 Nordlander, R. H. & Singer, M. Z. *Zellforsch.* **126**, 157-181 (1972).

Cross-tolerance between morphine and cholinergic blocking agents microinjected into anterior amygdala

WE have previously demonstrated tolerance to repeated applications of opiates microinjected directly into the amygdaloid region of the brain¹. Since morphine has been shown to inhibit the release of acetylcholine at some brain sites^{2,3}, as well as in the isolated guinea pig ileum^{4,5}, we compared the effect of morphine on amygdala bioelectric patterns with the effects of hemicholinium and scopolamine—two drugs that interfere with cholinergic synaptic activity in different ways. Tolerance that is produced with repeated administration of morphine is associated with cross-tolerance to hemicholinium and scopolamine.

Eighteen rats were anaesthetised with sodium pentobarbital 50 mg kg⁻¹, and bilateral cannula-recording electrode assemblies were implanted in the anterior amygdala. The permanently implanted cannula serves as a guide for

Fig. 1 Development of morphine tolerance to repeated daily microinjections (0.9 µl) of morphine sulphate 10 µg µl⁻¹ (M10) into the anterior amygdala of rat 460. Some recovery of the response was observed when the morphine concentration was increased three times (M30). ▲, M10 dose 1; ★, M10 dose 2; ×, M10 dose 4; ■, M10 dose 9; ●, M30 dose 10; —, no drug.

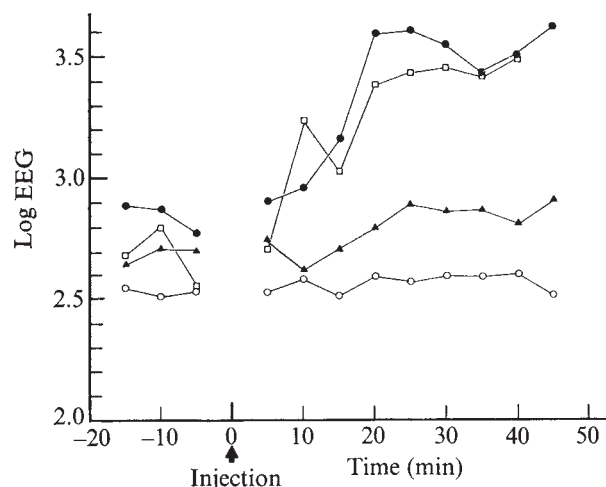
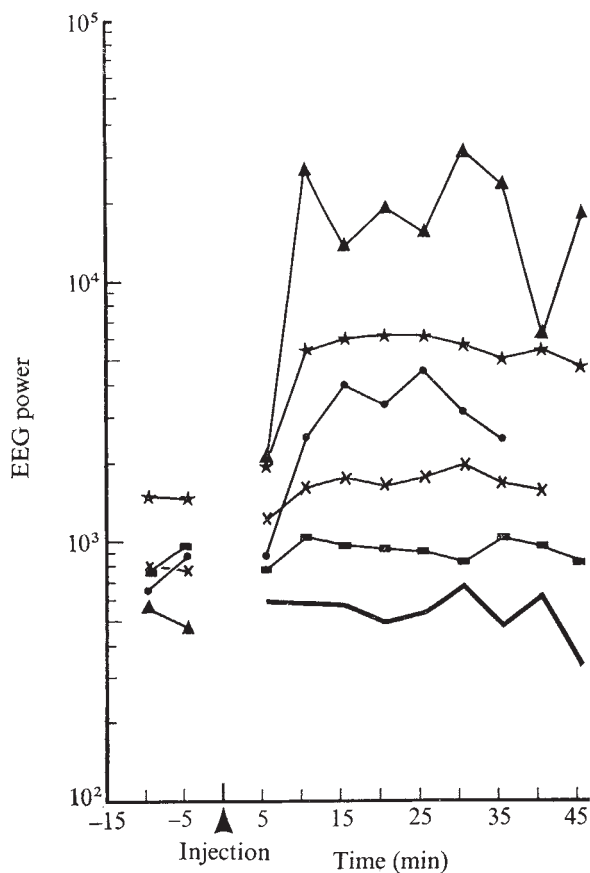


Fig. 2 Cross-tolerance between morphine 10 µg µl⁻¹ and hemicholinium 5 µg µl⁻¹ microinjected into left anterior amygdala of rat 488. Tolerance to hemicholinium was observed after ten consecutive microinjections. The hemicholinium-tolerant amygdala was also tolerant to morphine. ●, Morphine dose 1; □, hemicholinium dose 1; ▲, hemicholinium dose 10; ○, morphine dose 2 after hemicholinium dose 10.

smaller fluid delivery cannulae and as one pole of the bipolar recording assembly that monitors changes in bioelectrical activity at the injection site. All injections were delivered at a rate of 0.03 µl s⁻¹ for 30 s. In a previous report¹ we showed that analogue recordings from the injection site show spike and wave patterns in response to morphine sulphate but not to injections of saline and dextrophan, the ineffective (+)-isomer of levorphanol. Tolerance developed after repeated daily injections of morphine or levorphanol, whereas no change in morphine sensitivity was observed after repeated injections of saline. The morphine-responsive area was localised to a region ventrolateral to the anterior limb of the anterior commissure. This response to morphine was not observed at injection sites above or below this region or at medial thalamic loci. Haloperidol 6 µg µl⁻¹ had no effect when injected into morphine-responsive sites.

In the present experiment, the analogue recordings were subjected to digital conversion, and the root-mean-square value of EEG amplitude was computed for 5-s epochs taken at successive 5-min intervals before and after the unilateral injections.

To avoid confounding the drug response with high amplitude activity associated with sleep or drowsiness, the rats were maintained on a 22-h water-deprivation schedule. They were trained to consume their normal water intake (approximately 25 ml) by drinking from a water bottle spout that moved into an aperture at one end of the recording chamber on an aperiodic schedule, providing the rat with access to water for 3 s at a time. All the rats learned to consume their daily fluid intake in this manner before the recording sessions were started. Typically, each rat would sit quietly facing the aperture until it was time to drink. This procedure was very effective in providing a means of maintaining a steady EEG baseline throughout the recording session (for example, see Fig. 1).

Microinjections (0.9 µl) of morphine 10 µg µl⁻¹ caused the amplitude of bioelectric activity at the injection site to change from a mean of 567 µV² (s.d. 172) to a mean of 3,982 µV² (s.d. 1,925). This change was statistically significant ($P < 0.01$).

The progressive loss of sensitivity of the amygdala to repeated daily doses of morphine is shown in Fig. 1. Within 10 min after the first injection, the amplitude of the signal at this injection site increased to more than 10

times baseline values and persisted throughout the 45-min recording session. Tolerance, manifested by diminished responsiveness, developed in increments with each successive daily morphine treatment. In the experiment presented in Fig. 1, no observable response to morphine was seen on day 9. Increasing the concentration of morphine to $30 \mu\text{g } \mu\text{l}^{-1}$ on d 10, however, resulted in the reappearance of the morphine response from the tolerant amygdala (Fig. 1).

Cross-tolerance tests between morphine and cholinergic blocking drugs were carried out in 18 animals. In ten animals, tolerance to morphine was established first; in five, tolerance to scopolamine was established and morphine sensitivity was evaluated. Three were first made tolerant to hemicholinium and then tested with morphine. In every case, the first dose given was morphine, followed by a test dose of either scopolamine or hemicholinium.

In tests of cross-tolerance, in which morphine tolerance was first established, cross-tolerance to hemicholinium and scopolamine was found in eight out of ten cases. In two cases the response to scopolamine was reduced but still evident in morphine-tolerant subjects. Tolerance to morphine was observed in every case in which tolerance to scopolamine ($n=5$) and hemicholinium ($n=3$) had been previously established.

An experiment where hemicholinium tolerance was first established is shown in Fig. 2. First, the left amygdala of rat 488 was tested for sensitivity to morphine $10 \mu\text{g } \mu\text{l}^{-1}$ and then to hemicholinium $5 \mu\text{g } \mu\text{l}^{-1}$. A robust response was observed shortly after microinjection of each drug on

successive days. This injection site became tolerant to hemicholinium by the tenth dose of this drug. When the hemicholinium-tolerant injection site was retested for sensitivity to morphine, cross-tolerance to morphine was evident (Fig. 2).

Tolerance to scopolamine is demonstrated in Fig. 3a. The response to morphine before and after the development of scopolamine tolerance is shown in Fig. 3b. When morphine sensitivity was tested at this scopolamine-tolerant injection site, cross-tolerance to morphine was observed (dose 2, Fig. 3b).

Tolerance to morphine and cholinergic blocking drugs is not the result of an irreversible alteration of nerve cells in the anterior amygdala. After tolerance has been acquired, if the injection site is left alone for about 3 weeks, some recovery in the sensitivity to these drugs can be observed. This phenomenon is demonstrated in Fig. 3, in which tolerance to scopolamine and cross-tolerance to morphine was established in the right amygdala of rat 502.

When sensitivity was tested after a 25-d layoff, baseline recordings before injection remained stable, but tolerance to both scopolamine and morphine was no longer evident; the drug response was observed again ($n=3$).

In view of negative findings⁶⁻⁸, it is unlikely that cross-tolerance between opiates and cholinergic blocking drugs could have resulted from a change in the number or affinities of opiate receptor-binding sites.

It is possible that the chronic blockade of synaptic activity by morphine and the cholinergic blocking drugs used in this experiment caused a proliferation of postsynaptic neurotransmitter receptor sites⁹, producing both supersensitivity and cross-tolerance at the injection site. The relationship between disuse supersensitivity and tolerance has been discussed in detail by Jaffe and Sharpless¹⁰, and has been amply demonstrated^{5,11,12}.

Although cross-tolerance between systemic injections of morphine and muscarinic blocking drugs has been shown previously¹³, this is the first demonstration of symmetrical cross-tolerance between morphine and cholinergic blocking drugs microinjected directly into a discrete brain region.

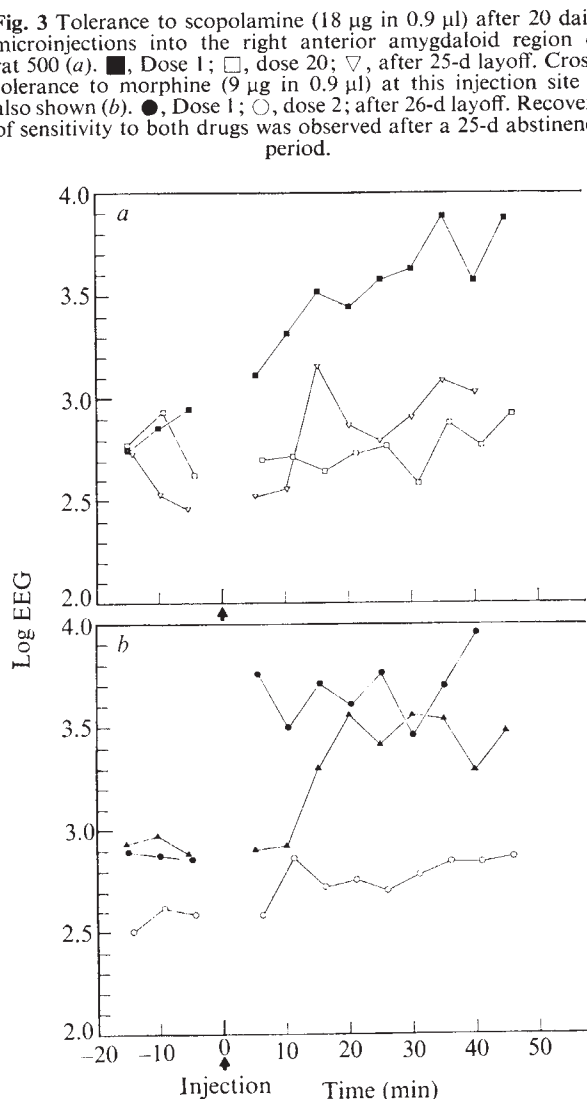
I thank Mr Jack F. Lee for assistance. This research was supported in part by the USPHS.

HERMAN TEITELBAUM

Behavioral Sciences Department,
Armed Forces Radiobiology Research Institute,
Bethesda, Maryland 20014

Received 7 January; accepted 17 March 1977.

- 1 Teitelbaum, H., Blosser, J. C. & Catravas, G. N. *Nature* **260**, 158-159 (1976).
- 2 Beleslin, D. & Polak, R. I. *J. Physiol., Lond.* **117**, 411-419 (1965).
- 3 Jhamandas, K., Pinsky, C. & Phillips, J. W. *Nature* **228**, 176-177 (1970).
- 4 Paton, W. D. M. *Br. J. Pharmac. Chemother.* **12**, 119-127 (1957).
- 5 Shoham, S. & Weinstock, M. *Br. J. Pharmac.* **52**, 597-603 (1974).
- 6 Pert, C. B. & Snyder, S. H. *Science* **179**, 1011-1014 (1973).
- 7 Pert, C. B., Pasternak, G. & Snyder, S. H. *Science* **182**, 1359-1362 (1973).
- 8 Klee, W. A. & Streaty, R. A. *Nature* **248**, 61-63 (1974).
- 9 Collier, H. O. J. *Nature* **220**, 228-231 (1968).
- 10 Jaffe, J. H. & Sharpless, S. K. *Proc. Ass. Res. nerv. ment. Dis.* **46**, 226-246 (1968).
- 11 Friedman, M. J., Jaffe, J. H. & Sharpless, S. K. *J. Pharmac. exp. Ther.* **167**, 45-55 (1969).
- 12 Wahlstrom, G. & Ekwall, T. *Eur. J. Pharmac.* **38**, 123-129 (1976).
- 13 Pert, A. & Maxey, G. *Psychopharmacologia Berl.* **44**, 139-145 (1975).



Electron spin resonance study of membrane protein alterations in erythrocytes in Huntington's disease

HUNTINGTON'S disease (HD), a degenerative disease of the central nervous system inherited as an autosomal dominant trait, is characterised clinically by progressive choreiform movements and dementia¹. Pathological changes include atrophy, severe cell loss and gliosis in the caudate nucleus and putamen and to a lesser extent the globus pallidus and cerebral cortex². The aetiology and pathogenesis of this disease are not understood. Although HD is considered a

defect of the basal ganglia and cerebral cortex, we report here evidence obtained by electron spin resonance (ESR) techniques (methods reviewed in ref. 3) which suggests HD may have more widespread membrane involvement.

Understanding of the basic biochemical defect in HD has not progressed greatly in recent years. Early biochemical studies performed in HD have been reviewed⁴. More recent studies have shown that the inhibitory synaptic transmitter γ -aminobutyric acid (GABA) and a related dipeptide, homocarnosine, were reduced in autopsied HD basal ganglia⁵. Reduced activity of glutamic acid decarboxylase, the enzyme involved in the synthesis of GABA, was found in post-mortem basal ganglia of HD patients⁶. Choline acetyltransferase, the biosynthetic enzyme for acetylcholine was also reported to be diminished⁶. Other studies have shown elevated levels of ribosomal proteins⁷ and lipofuscin pigment⁸ in HD neurons.

One of the major obstacles to biochemical studies of HD is that the primary alterations which give rise to its characteristic symptoms are found in the basal ganglia and cerebral cortex. Consequently, the availability of significant amounts of fresh, repetitively obtainable, useful specimens for study has been limited. Other uncontrollable factors such as delay in obtaining autopsy tissue, use of various drugs by patients, and super-imposed ageing changes or other pathological alterations have limited the value of such tissue and the enthusiasm of research in this area.

Similar considerations of sample purity and secondary biochemical changes on denervation of muscle led to the study of erythrocyte membranes in several inherited neuromuscular diseases. Our previous spin labelling studies⁹⁻¹³ have suggested that myotonic and Duchenne muscular dystrophy and congenital myotonia are diffuse membrane diseases even though muscle is the primary tissue in which symptoms are expressed. An increased membrane fluidity associated with myotonic states⁹⁻¹² and alterations in the membrane protein conformation and/or organisation in dystrophic states¹³⁻¹⁵ were suggested by these spin labelling studies.

We have performed similar ESR experiments with erythrocyte membranes from normal and HD patients. The principal spin label used was 2,2,6,6-tetramethylpiperidin-1-oxyl-4-maleimide (MAL-6) (Syva Associates). MAL-6 has been shown to covalently bind to sulphhydryl (SH) groups although a small amount of amino group binding may also occur¹⁶. Fresh, heparinised blood was obtained from 10 patients with well-documented HD from 10 different families. Four of the patients were in the very early stages of the disease, three were in the mid-stages, and three were in the bed-ridden, late stages. Seven of the 10 patients were ambulatory and active. Five of the HD patients were on a regular at-home diet; the other five were on a regular hospital diet. Two of the HD patients were on no medication, two were on diazepam, three were on a butyrophenone and a phenothiazine, and three were on an assortment of medication. Individuals without clinical manifestations or family history of inherited neurological diseases served as controls. Additional ESR studies were undertaken on erythrocyte membranes from patients with non-neurological disorders who were on a butyrophenone and a phenothiazine or diazepam. Intact erythrocytes and erythrocyte membranes (ghosts) were obtained and the spin labelling performed as previously described¹³ except a 1:25 weight ratio of spin label to membrane protein was used. Magnetic resonance experiments were performed on a Magnion-Ventron MVR-9X ESR spectrometer using a quartz aqueous sample cell.

A typical spectrum of erythrocyte ghost membranes labelled with MAL-6 is shown in Fig. 1. Spectra similar to that of Fig. 1 have been previously described as reflective of at least two classes of membrane protein SH groups: one strongly immobilised and the other weakly im-

mobilised¹³⁻¹⁹. The ratio of the spectral amplitude of MAL-6 attached to weakly immobilised SH groups (W) to that of MAL-6 attached to strongly immobilised SH groups (S) is a convenient monitor of protein organisational and/or conformational changes in membranes¹³⁻¹⁹.

The mean W/S ratio values of controls and HD erythrocyte membranes were compared by a two-way analysis of variance²⁰ (Table 1). This two-tailed method of statistical analysis minimises possible fluctuations from day to day between separate experimental values which can often occur with biological samples. P is the significance of the difference of mean values of $(W/S)_{\text{control}}$ and $(W/S)_{\text{HD}}$ calculated using a two-way analysis of variance. The null hypothesis used was that HD membranes are not different in their physical state compared with that of normal controls. The difference of $(W/S)_{\text{control}}$ and $(W/S)_{\text{HD}}$ is significantly less than zero, suggesting an altered conformation and/or organisation of membrane proteins in erythrocytes in HD. In each of the separate experiments represented by the results in Table 1, $(W/S)_{\text{control}}$ was less than $(W/S)_{\text{HD}}$. No alteration in sulphhydryl group content of HD could be demonstrated within the sensitivity of the analytical method used²¹.

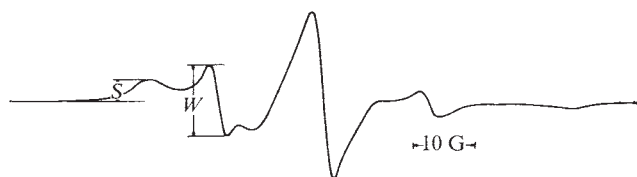


Fig. 1 Typical ESR spectrum of normal human erythrocyte membrane ghosts labelled with MAL-6. The amplitudes of the $M_I = +1$ weakly and strongly immobilised lines are indicated by W and S , respectively.

The W/S ratio was not altered from control values in erythrocyte ghosts from non-HD patients on diazepam. This parameter of MAL-6 was actually smaller than control values (3.2 compared with 3.7) in erythrocyte membranes from patients without HD who were on a butyrophenone and a phenothiazine. This change is in the opposite direction of the results in HD membranes (Table 1). Other workers have previously reported decreased W/S ratios of MAL-6 in bovine erythrocyte membranes on incubation with phenothiazine derivatives¹⁹. These findings together with the fact that some of our HD patients were on no medication, suggest that the increased values of W/S in HD (Table 1) are not simply due to drug effects. In addition, the clinical manifestations of HD in the patients in this study ranged from very early mild stages to severe, bed-ridden, late stages. Most were at home, active, and on a regular, normal diet. That the W/S ratio was increased in HD compared with normal controls in each experiment suggests that the current results are not due to effects of a particular stage of the disease, diet, or physical activity.

An alteration in the physical state of membrane proteins in erythrocytes in HD has been suggested by these experiments (Table 1). These results may be due to an altered enzyme or other membrane protein, altered amounts of membrane constituents, or altered constituent organisation. An additional possibility may be the presence of a membrane-bound moiety in HD, not found in controls. Experiments designed to discern these possibilities are in progress.

A difference in the conformation and/or organisation of membrane proteins in the basal ganglia like that found in erythrocytes in the present study may possibly give rise to the altered enzymatic behaviour observed in HD (refs 5, 6). Alternatively, the reduced activity of these enzymes in

Table 1 Comparison of ESR spectral amplitudes of MAL-6 attached to weakly immobilised SH groups (W) with that of MAL-6 attached to strongly immobilised SH groups (S) in control and Huntington's disease (HD) erythrocyte membranes

Mean $\pm$ s.e.m. $n = 10$	(W/S) _{control} 3.73 $\pm$ 0.11	(W/S) _{HD} 4.26 $\pm$ 0.11
	$P < 0.005$	

* P value calculated by a two-way analysis of variance using the null hypothesis that the respective means are equal.

HD may be a secondary consequence of an altered milieu in which they are located. The relationships of the present results to the molecular mechanisms by which the pathological and clinical manifestations of HD arise are unclear, but the alterations in the physical state of erythrocyte membranes which are outside the central nervous system suggest that this disease may be associated with a general membrane abnormality.

This work was supported by the Muscular Dystrophy Association of America, a Frederick Gardner Cottrell Grant from the Research Corporation, the Research Foundation and Graduate School of the University of Kentucky, and the Sanders-Brown Ageing Center. We thank Marcia Butterfield, Carole Bynum and Kim Hisle for technical assistance and Dr H. D. Jameson for help with patient referral.

D. ALLAN BUTTERFIELD*
JAMES Q. OESWEIN

Department of Chemistry,
University of Kentucky,
Lexington, Kentucky 40506

WILLIAM R. MARKESBERY

Departments of Neurology and Pathology,
University of Kentucky Medical Center,
Lexington, Kentucky 40506

Received 3 January; accepted 12 April 1977.

* To whom reprint requests should be addressed.

- Merritt, H. M. *A Textbook of Neurology* 5th edn 458–462 (Lea and Febiger, Philadelphia, 1973).
- Dreese, M. J. & Netsky, M. G. in *Pathology of the Nervous System*, 1 (ed. Minkler, J.) 1186–1193 (McGraw-Hill, New York, 1968).
- Spin Labelling—Theory and Applications* (ed. Berliner, L. J.) (Academic, New York, 1976).
- Barbeau, A. *Adv. Neurol.* 1, 473–516 (1973).
- Perry, T. L., Hansen, S. & Kloster, M. N. *Engl. J. Med.* 288, 337–342 (1973).
- Bird, E. D. & Iversen, L. L. *Brain* 97, 457–472 (1974).
- Iqbal, L., Tellez-Nagel, I. & Iqbal, I. G. *Brain Res.* 76, 178–184 (1974).
- Tellez-Nagel, I., Johnson, A. B. & Terry, R. D. *J. Neuropath. exp. Neurol.* 33, 308–332 (1974).
- Butterfield, D. A., Chesnut, D. B., Roses, A. D. & Appel, S. H. *Proc. natn. Acad. Sci. U.S.A.* 71, 909–913 (1974).
- Butterfield, D. A., Roses, A. D., Cooper, M. L., Appel, S. H. & Chesnut, D. B. *Biochemistry* 13, 5078–5082 (1974).
- Butterfield, D. A., Chesnut, D. B., Appel, S. H. & Roses, A. D. *Nature* 263, 159–161 (1976).
- Butterfield, D. A. & Watson, W. E. *J. membr. Biol.* 32, 165–176 (1977).
- Butterfield, D. A., Roses, A. D., Appel, S. H. & Chesnut, D. B. *Archs Biochem. Biophys.* 177, 226–234 (1976).
- Butterfield, D. A. *Acc. Chem. Res.* 10, 111–116 (1977).
- Butterfield, D. A. Submitted for publication (1977).
- Chapman, D., Barratt, M. D. & Kamat, V. B. *Biochim. biophys. Acta* 173, 154–157 (1969).
- Schneider, H. & Smith, I. C. P. *Biochim. biophys. Acta* 219, 73–80 (1970).
- Kirkpatrick, F. H. & Sandberg, H. E. *Archs Biochem. Biophys.* 156, 653–657 (1973).
- Holmes, D. E. & Piette, L. H. *J. pharmacol. exp. Therap.* 173, 78–84 (1970).
- Brownlee, K. A. *Statistical Theory and Methodology in Science and Engineering* (Wiley, New York, 1960).
- Sedlak, J. & Lindsay, R. H. *Analyt. Biochem.* 25, 152–205 (1968).

Marked enhancement of interferon production in 5-bromodeoxyuridine treated human lymphoblastoid cells

MANY human lymphoblastoid cells produce low levels of interferon spontaneously^{1–3} and this has been attributed to the presence of the Epstein-Barr virus (EBV) in such cells^{4–7}. No clear relationship has been established, however, between the presence of EBV and spontaneous interferon production⁸. Since the latent EBV genome contained in human lymphoblastoid cells can be activated by treatment of these cells with halogenated pyrimidines^{9,10} or inhibitors of protein synthesis¹¹ we determined the effect of 5-bromodeoxyuridine (BrdU) on interferon production in these cells in relation

to the induction of EBV. Previous studies on the effect of halogenated pyrimidines on interferon production have yielded conflicting results ranging from no effect^{12–14} to inhibition¹⁵, or slight stimulation^{15,16}. We report here that BrdU markedly increases both spontaneous and virus-induced interferon production in three human lymphoblastoid cell lines, without any apparent correlation with EBV induction.

The four human lymphoblastoid cell lines studied were chosen for their different characteristics in respect of origin, expression of EBV specified antigens, susceptibility to BrdU induction, and the capacity to produce interferon spontaneously. As reported by others^{4,5}, we found that Namalwa cells produce low levels of interferon spontaneously when maintained at a concentration of 10⁷ cells per ml for 24 h (Table 1). Pretreatment of exponentially multiplying cultures of Namalwa cells with BrdU markedly increased the titre of autogenous interferon produced. Maximum titres of spontaneous interferon (160–320 reference units per ml) were obtained after 72 h treatment with BrdU 25 μ g ml⁻¹ (Table 1). (No loss of viability was observed in BrdU treated cultures although the rate of cell multiplication was reduced.)

The spontaneous interferon produced in BrdU-treated cultures of Namalwa cells fulfilled the usual criteria for acceptance of a viral inhibitor as an interferon¹⁷ and was neutralised by a sheep anti-human leukocyte interferon serum. Spontaneous interferon production by Namalwa cells seemed to occur in the absence of EBV induction since no early antigen (EA) or virus capsid antigen (VCA) could be detected by immunofluorescence in either control or BrdU-treated cultures, in accord with other reports^{18,19}.

Raji cells, however, do express EA after treatment with BrdU (refs 17, 18), but have been reported not to produce interferon spontaneously^{2,4,5}. Similarly, we found that no spontaneous interferon was produced in untreated cultures of Raji cells even after 100-fold concentration of the culture supernatants (Table 1). Spontaneous interferon was detected in cultures of Raji cells after 72 h treatment with BrdU, however (Table 1). The spontaneous interferon was neutralised by a sheep antihuman leukocyte interferon serum.

BrdU also increased the amount of interferon produced by Namalwa and Raji cells in response to induction with Newcastle disease virus (NDV). Maximum titres were obtained after either 72 h treatment with BrdU 25 μ g ml⁻¹ or after 24 h treatment with BrdU 100 μ g ml⁻¹ (Table 2). (Treatment with BrdU 100 μ g ml⁻¹ for more than 24 h resulted in considerable cell toxicity.) Treatment of Namalwa cells with BrdU increased, by up to 32-fold, the titre of interferon produced on induction with NDV. A median increase of 8-fold in interferon titre was obtained in a total of 13 determinations (Table 2). Similarly, treatment of Raji cells with BrdU for 48–72 h gave a median increase of 33-fold in the titre of virus-induced interferon production (Table 2). The titres of both spontaneous and virus-induced interferon from BrdU treated cultures remained at the same high levels even when treated cells were subsequently cultivated in the absence of BrdU for 72 h before interferon induction. The continued presence of BrdU during the period of interferon induction did not affect the titre of either spontaneous or virus induced interferon obtained (experiment 4, Table 2). Treatment of Namalwa cells with 5-iododeoxyuridine (IrdU) gave a similar increase to that obtained with BrdU, in the titre of both spontaneous and virus-induced interferon, even though IrdU is a more efficient inducer of latent virus^{19,20}.

Although the mechanism of the enhancement of interferon production by BrdU is unknown, potentiation of interferon production is maximal after prolonged treatment of exponentially multiplying cultures with BrdU, conditions which would be expected to result in incorporation of BrdU into cellular DNA. In addition, the enhancement of

Table 1 Effect of BrdU on spontaneous interferon production and early antigen expression in human lymphoblastoid cells

Cell line	Time of treatment (h)	Interferon titre* (reference units per ml)		Frequency of EA-positive cells per 50,000	
		Control	BrdU treated	Control	BrdU treated
Namalwa Experiment 1	5	15	40	0	0
	24	10	40	0	0
	48	20	80	0	0
	72	10	160	0	0
Raji Experiment 2	72	15	320	0	0
Raji Experiment 1	26	< 5	< 5	4	79
	48	< 5	< 5	3	246
	72	< 5	30	1	111
	72	< 10†	240†	1	146
BJAB Experiment 2	72				
BJAB Experiment 1	24	< 5	< 5	0	0
	24	< 10	120	0	0
Experiment 2‡					

Lymphoblastoid cells were cultivated in RPMI 1640 medium with 10% foetal calf serum (GIBCO) in stirred suspension culture, for the times indicated, with or without BrdU ($25 \mu\text{g ml}^{-1}$). Each culture was then centrifuged (800g, 15 min), washed once, and the cells resuspended at a concentration of 10^7 cells per ml in the same medium but without BrdU. After 24 h incubation at 37°C in stirred suspension culture, the culture supernatant was harvested by centrifugation (800g, 15 min) and assayed for interferon content after 10-fold concentration with Aquacide (Calbiochem) and dialysis against phosphate buffered saline, pH 7.4. EBV early antigen (EA) was determined on cells at the same time as the culture supernatants were harvested for the assay of interferon. EA was determined on acetone-fixed smears by indirect immunofluorescence using human sera and fluorescein-conjugated goat anti-human IgG globulin (Huntingdon Laboratories, U.S.A.).

Virus-induced interferon production was determined in the same manner as described in Table 1 with the exception that NDV was added to the culture at the concentration indicated. All samples were dialysed at pH 2.0 for 7 d to inactivate residual NDV before assay for interferon.

*Interferon was assayed by inhibition of the cytopathic effect of 100 TCID₅₀ of vesicular stomatitis virus on monolayer cultures of human foreskin fibroblasts as previously described³². Interferon titre expressed as the reciprocal. All interferon titres are expressed in terms of the international reference preparation 69/19 (National Institute of Medical Research, London).

†Sample concentrated 100-fold.

‡To minimise toxicity and increase efficiency of incorporation into DNA, BrdU was added to a culture already multiplying exponentially. Samples concentrated 50-fold.

interferon production by BrdU was reduced when cells were treated with BrdU in the presence of added thymidine, or when BrdU treated cells were exposed to visible light. Incorporation of BrdU into cellular DNA is also known to be necessary for BrdU activation of EBV (refs 20–22). It seems unlikely, however, that EBV induction is responsible for the increase in either spontaneous or virus-induced interferon production in BrdU treated cultures, since this enhancement occurred in Namalwa cells in the absence of EBV induction. Namalwa cells, however, do contain two (probably incomplete) copies of the EBV genome²³. Therefore to exclude the possible involvement of EBV induction we investigated the effect of BrdU on interferon production in the human lymphoblastoid cell line BJAB which does not express the EBV-specified nuclear antigen EBNA and which is negative for EBV by nucleic acid hybridisation²⁴. Spontaneous inter-

feron was detected (after 50-fold concentration) in the culture supernatants from BrdU treated BJAB cells, but not in the supernatants from untreated control cultures (Table 1). The spontaneous interferon produced was neutralised by a sheep anti-human leukocyte interferon serum. There was also a clear cut increase in NDV-induced interferon production in BrdU treated BJAB cells (Table 2). No EA or VCA was detected either before or after treatment of BJAB cells with BrdU. These results strongly suggest that induction of EBV by BrdU is not responsible for the observed increase in interferon production in BrdU treated cultures.

Namalwa, Raji and BJAB are all B-lymphocyte derived cell lines^{18,24}. It was therefore of interest to determine the effect of BrdU on the T-lymphocyte cell line JM²⁵. The JM cell line did not produce interferon spontaneously either

Table 2 Effect of BrdU on virus-induced interferon production in human lymphoblastoid cells

Cell line	Time of treatment (h)	NDV concentration (HA units/ml)	Interferon titre* (reference units per ml)	
			Control	BrdU treated ($25 \mu\text{g ml}^{-1}$)
Namalwa				
	Experiment 1	24	1,280	19,200†
	Experiment 2	72	3,200	25,600
	Experiment 3	72	800	25,600
	Experiment 4	72	1,600	9,600
		25.6	2,400	19,200
		256‡	3,200	38,400
Raji		1,280	9,600	19,200
	Experiment 1	26	60	240
		48	320	4,800
		72	320	9,600
	Experiment 2	72	240	8,600
BJAB				
	Experiment 1	27	< 20	480
	Experiment 2	24	40	160

*Titre expressed as the reciprocal.

†Culture treated with BrdU $100 \mu\text{g ml}^{-1}$.

‡Culture not washed after removal of BrdU.

before or after treatment with BrdU. In addition, treatment of JM cell with BrdU ($25 \mu\text{g ml}^{-1}$) decreased the titre of interferon produced in response to induction with NDV. It remains to be determined whether the behaviour of JM cells is characteristic of T cells in general. Preliminary results suggest that BrdU also increases NDV-induced interferon production in a non-lymphoid Moloney sarcoma virus transformed mouse cell line (C 243-3 cells), but not in a line of human or rat fibroblasts.

BrdU uniformly incorporated into cellular DNA has been reported both to increase²⁶ and to selectively inhibit²⁷ the transcription of certain genes in mammalian cells. Interferon induction is sensitive to actinomycin D²⁸ and represents a process analogous to enzyme induction²⁹. Two possible explanations of our results would be that either BrdU increases the transcription of the interferon messenger or inhibits the transcription of an inhibitor of interferon synthesis.

Human lymphoblastoid cells (and in particular the Namalwa cell line) have received attention as a source of human interferon for clinical trials^{30,31}, since these cells can be cultivated in suspension culture in virtually limitless quantity.

Our results show that up to a 32-fold increase in interferon titre can be obtained by treatment of Namalwa cells with BrdU. Furthermore, higher interferon titres can be obtained from BrdU-treated cultures of Namalwa cells, inoculated with considerably less virus inducer than from untreated cultures induced with optimal amounts of NDV (Table 2, experiment 4). We believe that these results may therefore be of considerable importance for the large scale production of human interferon.

We thank Dr Gilbert Lenoir for helpful suggestions and for the gift of the BJAB and JM cell lines, Dr Guy de Thé for the EBV-specific human sera, Dr Kari Cantell for sheep anti-human leukocyte interferon serum, Dr Hans Strander for Namalwa cells, and Dr Pierre Verroust for Raji cells. This work was supported by the Délégation Ministérielle, Direction des Recherches et Moyens d'Essais and by the CNRS.

MICHAEL G. TOVEY
JACQUELINE BEGON-LOURS
ION GRESSER
ALAN G. MORRIS*

*Institut de Recherches Scientifiques sur le Cancer,
Villejuif, France*

Received 28 January; accepted 28 March 1977.

*Present address: Department of Biological Sciences, University of Warwick, Coventry, UK.

- 1 Henle, G. & Henle, W. J. *Bact.* **89**, 252-258 (1965).
- 2 Zajac, B. A., Henle, W. & Henle, G. *Cancer Res.* **29**, 1467-1475 (1969).
- 3 Swart, B. E. & Young, B. G. J. *natn. Cancer Inst.* **42**, 941-944 (1969).
- 4 Adams, A., Lidin, B., Strander, H. & Cantell, K. J. *gen. Virol.* **28**, 219-223 (1975).
- 5 Lidin, B. & Adams, A. *Intervirology* **5**, 205-215 (1975).
- 6 Epstein, M. A., Barr, Y. M. & Achong, B. G. *Wistar Inst. Symp. Monograph* **4**, 69-82 (1965).
- 7 Henle, G. & Henle, W. *Cancer Res.* **27**, 2442-2446 (1967).
- 8 Oxman, M. N. in *Interferons and Interferon Inducers* (ed. Finter, N. B.) 424-427 (North-Holland, Amsterdam, 1973).
- 9 Hampar, B., Derge, J. G., Martos, L. M. & Walker, J. L. *Proc. natn. Acad. Sci. U.S.A.* **69**, 78-82 (1972).
- 10 Gerber, P. *Proc. natn. Acad. Sci. U.S.A.* **69**, 83-85 (1972).
- 11 Hampar, B., Lenoir, G., Nonoyama, M., Derge, J. G. & Chang, S. Y. *Virology* **69**, 660-668 (1976).
- 12 Levy, H. B., Axelrod, D. & Baron, S. *Proc. Soc. exp. Biol. Med.* **118**, 1013-1014 (1965).
- 13 Coppey, J. & Muel, B. *Int. J. radiat. Biol.* **17**, 431-437 (1970).
- 14 Holmes, A. W., Gilson, J. & Deinhardt, F. *Virology* **24**, 229-232 (1964).
- 15 Donikian, M. A. & Stewart, R. C. *Bact. Proc. Abstr.* **157**, 144 (1964).
- 16 Swetly, P. J. *Virol.* **17**, 27-32 (1976).
- 17 Lockart, R. Z. in *Interferons and Interferon Inducers* (ed. Finter, N. B.) 11-27 (North-Holland, Amsterdam, 1973).
- 18 Nyormoi, O., Klein, G., Adams, A. & Dombos, L. *Int. J. Cancer* **12**, 396-408 (1973).
- 19 Klein, G. & Dombos, L. *Int. J. Cancer* **11**, 327-337 (1973).
- 20 Teich, N., Lowy, D. R., Hartley, J. W. & Rowe, W. P. *Virology* **51**, 163-173 (1973).
- 21 Hampar, B. *et al. Nature new Biol.* **244**, 214-217 (1973).
- 22 Hampar, B., Tanaka, A., Nonoyama, M. & Derge, J. *Proc. natn. Acad. Sci. U.S.A.* **71**, 631-633 (1974).
- 23 Pritchett, R., Pedersen, M. & Kieff, E. *Virology* **74**, 227-231 (1976).
- 24 Klein, G. *et al. Proc. natn. Acad. Sci. U.S.A.* **71**, 3283-3286 (1974).
- 25 Schwenk, H. U. & Schneider, U. *Blut* **31**, 299-306 (1975).
- 26 Price, P. M. *Biochim. biophys. Acta* **447**, 304-311 (1976).
- 27 Stellwagen, R. H. & Tomkins, G. M. *Proc. natn. Acad. Sci. U.S.A.* **68**, 1147-1150 (1971).

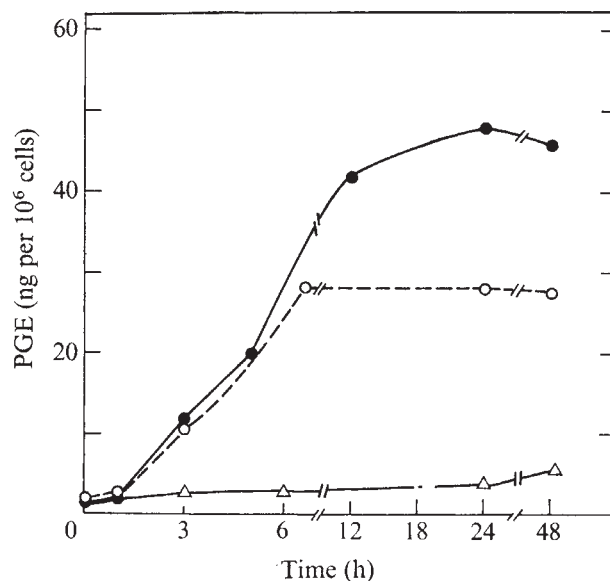
- 28 Heller, E. *Virology* **21**, 652-656 (1963).
- 29 Ng, M. H. & Vilcek, J. *Adv. Protein Chem.* **26**, 209-234 (1972).
- 30 Strander, H., Mogensen, K. E. & Cantell, K. J. *clin. Microbiol.* **1**, 116-117 (1975).
- 31 Tovey, M. G., Begon-Lours, J. & Gresser, I. *Proc. Soc. exp. Biol. Med.* **146**, 809-815 (1974).
- 32 Gresser, I., Bandu, M. T., Brouty-Boye, D. & Tovey, M. G. *Nature* **251**, 543-545 (1974).

Stimulation of prostaglandin E production in cultured human fibroblasts by poly(I) · poly(C) and human interferon

PROSTAGLANDINS (PGs) have emerged as one of the principal mediators of the inflammatory process¹⁻³. Elevation of tissue concentration of PGs was noted following inflammation^{3,4}, or treatment with various hormones^{5,6}, cyclic nucleotides (ref. 7 and U.Z., B. Strulovici and H.R.L., in preparation), serum⁸, drugs^{8,9} and antigen¹⁰. The beneficial effects of anti-inflammatory drugs have been correlated with their ability to suppress PG production either by curtailing precursor availability (glucocorticosteroids^{11,12}) or by inhibiting PG synthetase activity (non-steroidal drugs¹). Viral infections are associated with interferon production¹³ and with inflammation¹⁴. Little is known about the possible involvement of interferon in the inflammatory reaction, though it has been suggested that interferon may have anti-inflammatory properties¹⁵. It has been shown that an interferon inducer, double-stranded polyinosinate-polycytidylate [poly(I)·poly(C)], as well as human interferon, stimulate hyaluronic acid production by human synovial fibroblasts in culture¹⁶. Overproduction of hyaluronic acid is known to be associated with the inflammatory process¹⁶; the effect is prevented by cortisol or indomethacin¹⁶. We report here the stimulation by poly(I)·poly(C) and by human interferon of prostaglandin E (PGE) production by human synovial and foreskin fibroblasts in culture. Cortisol inhibited this effect.

Fibroblasts derived from synovial membranes of patients undergoing surgery for meniscus tear were grown in culture using methods described by Castor *et al.*¹⁷. Human fibroblast interferon (2×10^4 U ml⁻¹) was prepared from foreskin fibro-

Fig. 1 Time course of stimulation of PGE production in cultured synovial fibroblasts by poly(I)·poly(C) and human interferon. Synovial fibroblasts were sown (about 4×10^5 cells per ml) in a medium containing Parker CMRL-1066 (80%), heat-inactivated human serum (10%) and foetal calf serum (10%), supplemented by L-glutamine, penicillin and streptomycin, and grown as monolayers. Samples of the medium were withdrawn after the time-intervals indicated for PGE determination. Δ , Controls; $\bullet$, poly(I)·poly(C) added to the medium ($100 \mu\text{g ml}^{-1}$); $\circ$, human fibroblast interferon added (100 U ml⁻¹).



blasts (FS4) by the method of Havell and Vilcek¹⁸. Partially purified human lymphoblastoid interferon (purification about 200-fold) was prepared as described by Anfinson *et al.*¹⁹. Poly(I):poly(C) (Miles) or interferon were incubated with the synovial fibroblast and foreskin cell cultures for various times and the accumulation of prostaglandins of the E group in the medium was determined by radioimmunoassay as previously described²⁰.

Both poly(I):poly(C) ($100 \mu\text{g ml}^{-1}$) and human interferon (100 U ml^{-1}) stimulated PGE production by synovial fibroblasts: increased accumulation of PGE in the medium was noted within 3 h and a peak was reached at 24 h (Fig. 1). Mouse interferon did not stimulate PGE production in these human cells. The lowest effective concentration of poly(I):poly(C) was $10 \mu\text{g ml}^{-1}$ and the response was maximal at $20\text{--}100 \mu\text{g ml}^{-1}$ (Fig. 2). Single-stranded polyinosinate (poly I) or polycytidylate (poly C) or polyadenylate:polyuridylylate [poly(A):poly(U)] did not stimulate PGE production when added to cultured synovial fibroblasts at $100 \mu\text{g ml}^{-1}$. Poly(I):poly(C) did not induce PGE production

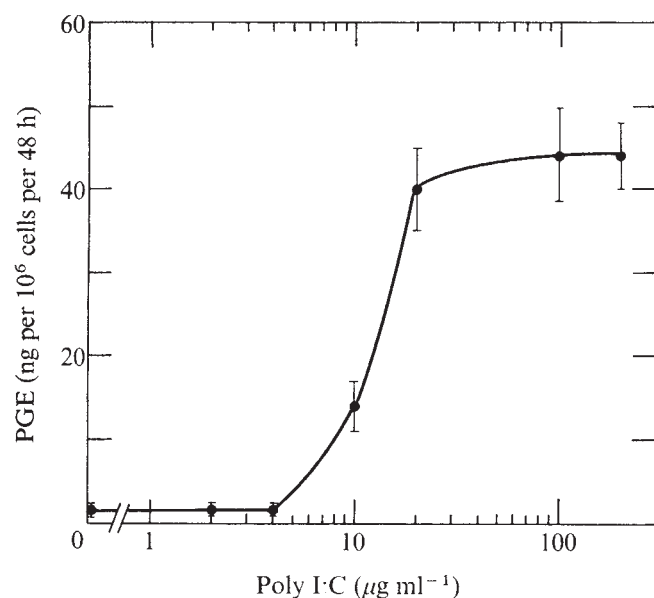


Fig. 2 Dose-response curve of stimulation of PGE production in cultured human synovial fibroblasts by poly(I):poly(C). PGE concentration in the medium was determined by radioimmunoassay. Vertical bars, s.e.m. ($n = 8$). For experimental details see Fig. 1 legend.

in cultured human peripheral blood lymphocytes, which are known not to respond to double-stranded RNA with interferon production. In contrast, human interferon and poly(I):poly(C) stimulated PGE production in two lines of human foreskin fibroblasts tested (FS7 and FS4). Stimulation was proportional to the amount of interferon added (Fig. 3). Both fibroblast and lymphoblastoid human interferon stimulated PGE production in foreskin cells. Addition of partially purified lymphoblastoid interferon to the medium (100 U ml^{-1}) elicited a 40-fold stimulation of PGE. Cycloheximide ($10 \mu\text{g ml}^{-1}$) and actinomycin D ($2 \mu\text{g ml}^{-1}$) prevented the stimulation by poly(I):poly(C) of PGE formation (data not shown). The stimulatory action of poly(I):poly(C) and of interferon on PGE production by foreskin fibroblast cultures was also abolished by concomitant addition of cortisol ($2 \mu\text{g ml}^{-1}$) to the medium (Fig. 4). Both poly(I):poly(C) and human interferon had a moderate (30%) inhibitory effect on synovial fibroblast proliferation and cortisol prevented this effect (data not shown).

This is the first report to show that interferon is capable

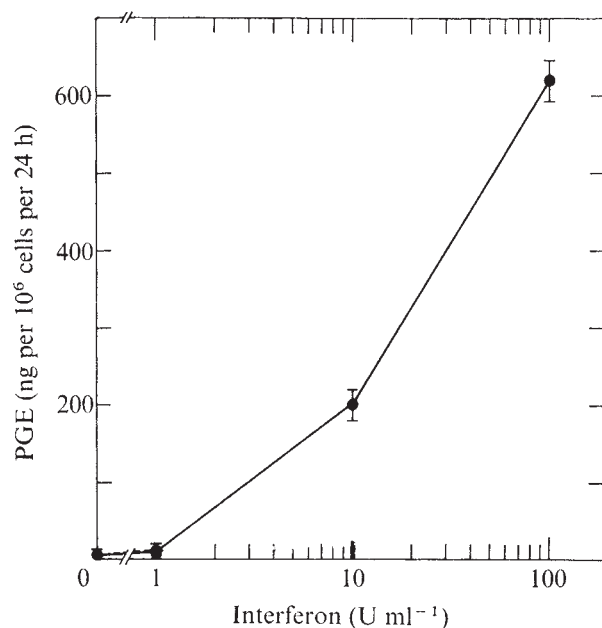
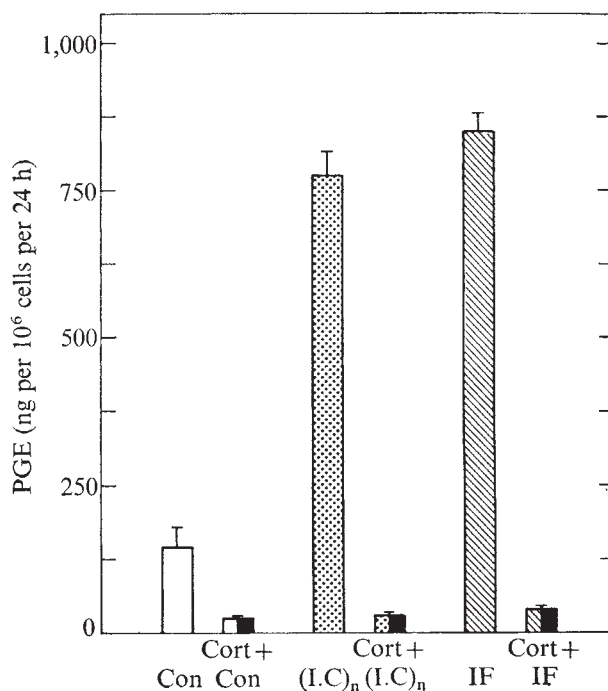


Fig. 3 Dose-response curve of stimulation of PGE production by human foreskin fibroblast interferon in human foreskin fibroblast cultures. The cells (2×10^5 cells per ml) were sown in Eagle's MEM medium containing 5% heat-inactivated foetal calf serum and penicillin and streptomycin and incubated for 24 h at 37°C with various doses of interferon. PGE was determined by RIA. Bars represent mean $\pm$ s.e.m.

of stimulating the production of PGE, an established mediator of inflammation¹⁻³. This effect shares the known species specificity of interferon action, since only interferons of human origin, but not mouse interferon, induced PGE synthesis in human fibroblast cells. The efficacy of partially purified lymphoblastoid interferon makes it unlikely that the observed effect was due to contaminating

Fig. 4 Prevention by cortisol (Cort; $2 \mu\text{g ml}^{-1}$) of the stimulatory effect of poly(I):poly(C) [(I.C.)_n; $100 \mu\text{g ml}^{-1}$] and human fibroblast interferon (IF; 100 U ml^{-1}) on PGE production by human foreskin fibroblasts. Con, control. Other experimental details as for Fig. 3.



material. This study shows that poly(I)-poly(C), which induces interferon formation in synovial and foreskin fibroblasts¹⁶⁻¹⁸, is also capable of stimulating PGE production. Poly(A)-poly(U) and single-stranded poly(I) or poly(C), which do not induce interferon formation, are not active in this respect. The induction of PGE synthesis by either interferon or poly(I)-poly(C) has a latency of about 3 h. During this time both RNA and protein synthesis are required.

Cortisol, a well known anti-inflammatory drug, which is able to reduce PGE production by inflamed synovial membrane^{12,21}, abolished PGE production induced by both interferon and poly(I)-poly(C). We suggest that interferon may have a role in propagating the inflammatory effect of viral infections through prostaglandins, which have been shown to participate in the body's general defence mechanism²². It is of interest that both glucocorticoids and aspirin seem to enhance the pathogenic effects of viral infections^{23,24}. The present findings are difficult to reconcile with those of Koltai and Mecs¹⁵, who have attributed to the interferon inducer, poly(I)-poly(C), an inhibitory effect on inflammation.

Another common effect of interferon and prostaglandins of the E type is their potent inhibition of the replication of several cell types²⁵⁻²⁷. Cortisol and indomethacin, which inhibit PGE production^{1,11,12,21}, stimulated tumour cell replication²⁶ and prevented the inhibitory effect of interferon on synovial fibroblast growth. The significance of prostaglandin synthesis in the mode of action of poly(I)-poly(C) and interferon as antiviral and anti-tumour agents is not yet clear and is under further study.

M. YARON
I. YARON

Department of Physical Medicine and
Rheumatology,
Rokach (Hadassah) Hospital,
Tel Aviv

D. GURARI-ROTMAN
M. REVEL

Department of Virology,

H. R. LINDNER
U. ZOR

Department of Hormone Research,
Weizmann Institute of Science,
Rehovot, Israel

Received 11 March; accepted 6 April 1977.

- ¹ Vane, J. R. *Nature new Biol.* 231, 232-235 (1971).
- ² Di Rosa, M., Giroud, J. P. & Willoughby, D. A. *J. Pathol.* 104, 15-29 (1971).
- ³ Floman, Y., Okon, E. & Zor, U. *Clin. Orthop. Rel. Res.* (in the press).
- ⁴ Ferreira, S. H., Moncada, S. & Vane, J. R. in *Prostaglandin Synthetase Inhibitors* (eds Robinson, H. J. & Vane, J. R.) 175-187 (Raven, New York, 1974).
- ⁵ Ramwell, P. W., Shaw, J. E., Douglas, W. W. & Poisner, A. M. *Nature*, 210, 273-274 (1966).
- ⁶ Alexander, R. W. & Gimbrone, M. A. Jr, *Proc. natn. Acad. Sci. U.S.A.* 73, 1617-1620 (1976).
- ⁷ Hamprecht, B., Jaffe, B. M. & Philpott, G. W. *FEBS Lett.* 36, 193-198 (1973).
- ⁸ Hong, S.-C. L., Polsky-Cynkin, R. & Levine, L. *J. biol. Chem.* 251, 776-780 (1976).
- ⁹ Robinson, D. R., Smith, H., McGuire, M. B. & Levine, L. *Prostaglandins* 10, 67-85 (1975).
- ¹⁰ Webb, D. R. & Osheroff, P. L. *Proc. natn. Acad. Sci. U.S.A.* 73, 1300-1304 (1976).
- ¹¹ Gryglewski, R. J., Panzenko, B., Korbut, R., Grodzinska, L. & Ocetkiewicz, A. *Prostaglandins* 10, 343-356 (1975).
- ¹² Floman, Y. & Zor, U. *Prostaglandins* 12, 403-413 (1976).
- ¹³ Isaacs, A. & Lindemann, J. *Proc. R. Soc. B* 147, 258-267 (1957).
- ¹⁴ Smith, J. W. & Sanford, J. P. *Ann. Intern. Med.* 67, 651-659 (1967).
- ¹⁵ Koltai, M. & Mecs, E. *Nature* 242, 525-526 (1973).
- ¹⁶ Yaron, M., Yaron, I., Smetana, O., Eylan, E. & Herzberg, M. *Arthritis Rheumatism* 19, 1315-1320 (1976).
- ¹⁷ Castor, C. W., Prince, R. K. & Dorstewitz, E. L. *Lab. Invest.* 11, 703-713 (1962).
- ¹⁸ Havell, E. A. & Vilcek, J. *Antimicrob. Agents Chemother.* 2, 476-484 (1972).
- ¹⁹ Anfinson, C. B., Bose, S., Corley, L. & Gurari-Rotman, D. *Proc. natn. Acad. Sci. U.S.A.* 71, 3139-3142 (1974). Gurari
- ²⁰ Bauminger, S., Zor, U. & Lindner, H. R. *Prostaglandins* 4, 313-324 (1973).
- ²¹ Kantrowitz, F., Robinson, D. R., McGuire, M. B. & Levine, L. *Nature* 258, 737-739 (1975).
- ²² Collier, H. O. *J. Nature* 232, 17-19 (1971).
- ²³ Kilbourne, E. D. & Horsfall, F. L., Jr, *Proc. Soc. exp. biol. Med.* 77, 135-138 (1951).
- ²⁴ Stanley, E. D., Jackson, G. G., Panusarn, C., Rubenis, M. & Dirda, V. J. *Am. Med. Assoc.* 231, 1248-1251 (1975).
- ²⁵ Weinstein, Y., Brodeur, B. R., Melmon, K. L. & Merigan, T. C. *Immunology* (in the press).
- ²⁶ Santoro, M. G., Philpott, G. W. & Jaffe, B. M. *Nature* 263, 777-779 (1976).
- ²⁷ Johnson, G. S. & Pastan, I. *J. natn. Cancer Inst.* 47, 1357-1364 (1971).

Virus-like particles from both control and scrapie-affected mouse brain

Cho and Grieg¹ reported that 14-nm virus-like particles could be extracted from the brains of mice in the late clinical stage of scrapie but not from the brains of young, uninoculated mice. The extraction procedure involved repeated fluorocarbon treatment followed by equilibrium banding on CsCl gradients. One band was obtained from extracts of normal brain at a density of 1.29-1.30 g cm⁻³ and two bands were obtained from scrapie brain extracts at densities of 1.29-1.30 g cm⁻³ and 1.33-1.34 g cm⁻³, respectively. Electron microscope studies showed that 14-nm particles were found mainly in the 1.33-1.34 g cm⁻³ fraction derived from scrapie brains. It was subsequently reported² that similar particles could be obtained at the same density after centrifuging extracts of scrapie hamster brain. This second study also showed that the exhaustive extraction procedure resulted in a large loss of infectivity, but the small amount of infectivity remaining sedimented mainly in the same position as the 14-nm particles. From these observations it was suggested that the 14-nm particles might be the causative agent of scrapie. Similar studies have been carried out at Compton during the last year in an attempt to repeat these findings with brain both from mice in the late clinical stage of infection and from sheep with natural scrapie. In none of these studies was a visible band seen at 1.33-1.34 g cm⁻³ after isopycnic centrifugation in CsCl. Electron microscopy studies of this fraction derived from scrapie mice showed 14-nm particles, resembling those originally reported, in one field only; the sample was rich in other particulate material common to control samples also. In order to resolve these different findings a collaborative study was arranged at Lethbridge in which scrapie mouse brain and brains of age-matched control mice (previously injected with normal mouse brain) from both Institutes were extracted. We report here that 14-nm particles can be visualised from both control and scrapie-affected mouse brain.

Four groups of mice were used. Two groups of female, random bred, Swiss white mice of the Animal Diseases Research Institute (Western) Lethbridge were inoculated intracerebrally with 0.03 ml of a 1% (wet weight) suspension of mouse brain in physiological saline; the control group was injected with a suspension of normal mouse brain and the scrapie group with a suspension of brain from clinically-affected mice. The other two groups were specific pathogen free, female, Compton white mice injected in an identical manner. The Lethbridge mice were infected with the Compton strain of mouse-adapted scrapie obtained from Dr W. J. Hadlow³ and the Compton mice were infected with the same strain⁴, passaged at Compton. Scrapie mice were killed in the late clinical stage of the disease (after 18 weeks) and the brains collected and stored frozen (-20 °C). Control brains were collected at the same time. The brain samples from Compton were coded by an independent observer before being taken to Lethbridge. The weights of the various brain pools used for extraction were as follows: 97 g of control brain and 80 g of scrapie brain from Compton, 123 g of control brain and 175 g of scrapie brain from Lethbridge. The method of extraction used was the same as originally described¹. After the extensive Freon extraction procedure, material equivalent to 12 g of original brain in a volume of 1.0 ml was centrifuged on a CsCl gradient in a Spinco 50.1 Ti Sw Rotor at 243,000g for 72 h.

After centrifugation of the Lethbridge control sample only one distinct band was seen in the CsCl gradient, at a density of 1.29-1.30 g cm⁻³, whereas the Lethbridge scrapie sample gave two bands, one identical to the control and an additional, faint band which was seen at a density of

1.33–1.34 g cm⁻³. Centrifugation of both Compton samples (normal and scrapie) gave less distinct bands with most of the material sedimenting to a density of 1.29–1.30 g cm⁻³. Ten equal fractions of 0.45 ml were collected from all four samples by syringes from the side of the tube and each fraction was subsequently examined under the electron microscope as described before¹ at both Compton and Lethbridge.

At Lethbridge spherical particles similar to those originally described by Cho and Greig¹ were seen at a density of 1.33–1.34 g cm⁻³ from both the control and both the scrapie extracts. The particles were morphologically similar from all four sources. At Compton very few particles were seen in the projected size range in any of the four samples; however, conditions of storage may have accounted to some extent for these different observations at the two centres. The evidence indicated that, where particles were seen, they were present in both scrapie and control preparations.

These findings suggest that the particles were either of host origin or, alternatively, represent an unknown species of virus which is present in both normal and scrapie-affected mice. The former possibility is considered more likely since similar particles have also been obtained from the brains of scrapie-affected hamsters² and goats (H.J.C. unpublished).

Since 14-nm particles can be obtained from both control and scrapie-affected brain, it seems that they are not specifically associated with scrapie. The scrapie infectivity reported to be present at a density of 1.33–1.34 g cm⁻³ may therefore be associated with some entity other than the 14-nm particles. Alternatively, it is possible that although the particles obtained from both control and scrapie mice were morphologically similar, there may be biochemical differences; for example the 14-nm particles from scrapie brain might harbour a scrapie-specific nucleic acid. This possibility can be tested by a detailed analysis of the biochemical components present in highly purified preparations of 14-nm particles from several sources. Some preliminary, immunological studies have already been carried out involving treatment of 14-nm particles from scrapie mouse brain with anti-mouse ferritin serum prepared in rabbits. Aggregation of the 14-nm particles was observed by electron microscopy indicating that the particles isolated from scrapie brain share common antigen(s) with mouse ferritin (H.J.C. and Darcel, unpublished).

We are grateful to the Multiple Sclerosis Society for the provision of funds to Dr G. D. Hunter, whom we thank for encouragement and advice. We thank Miss Carol Walker and Miss Janice Robinson for technical assistance and Miss S. Becker and Dr M. S. Shahrabadi in assisting with the electron microscopy. Dr L. D. Miller kindly provided the scrapie goat brains.

H. J. CHO
A. S. GREIG

Animal Diseases Research Institute (Western),
P.O. Box 640, Lethbridge,
Alberta T1J 3Z4, Canada

C. R. CORP
R. H. KIMBERLIN
R. L. CHANDLER
G. C. MILLSON

ARC Institute for Research on Animal Diseases,
Compton, Newbury,
Berkshire, UK

Received 9 March; accepted 19 April 1977.

¹ Cho, H. J. & Greig, A. S. *Nature* 257, 685–686 (1975).

² Cho, H. J. *Nature* 262, 411–412 (1976).

³ Eklund, C. M., Kennedy, R. C. & Hadlow, W. J. *J. infect. Dis.* 117, 15–22 (1967).

⁴ Chandler, R. L. *Res. vet. Sci.* 4, 276–285 (1963).

Role for nucleocapsid protein phosphorylation in the transcription of influenza virus genome

LIKE other minus-strand RNA viruses^{1–3}, influenza virus seemed to contain protein kinase as well as viral transcriptase in, or in association with, nucleocapsid structure⁴. The kinase phosphorylates NP-protein constituting the nucleocapsid, but none of the other virion proteins in *in vitro* reaction⁵. Since the influenza virus RNA serves as a template for transcription in the form of nucleocapsid in which the viral RNA is tightly packed in helical array of NP-protein^{7–9}, it is tempting to implicate the NP-protein phosphorylation in the control of viral transcription. We report here that the phosphorylation of NP-protein stimulates viral transcription, and that some conformational changes in the nucleocapsid may be involved in the stimulation of transcription.

Influenza A₂ virus (R1/57/57) was grown in embryonated eggs and purified as described previously⁶. None of the viral proteins was phosphorylated to a significant extent during virus growth in eggs⁶. The purified virus suspension was divided into two portions, one of which was phosphorylated with the endogenous protein kinase as described (legend to Fig. 1). In these conditions, only NP-protein of the virion proteins was phosphorylated and its phosphate-accepting sites were saturated within 20 min (ref. 6). The phosphorylated nucleocapsid was then isolated and made free of ATP (legend to Fig. 1). Since it was crucially important to confirm complete removal of ATP from the nucleocapsid, the other portion of the nucleocapsid preparation was treated in the same manner as the first, except that ATP was added at the end of incubation and then removed as above. This unphosphorylated preparation will be referred to as 'native nucleocapsid'. A separate experiment in which ¹⁴C-ATP was incubated with the nucleocapsid and then removed by the same procedure, showed that a residual amount of free ATP, if any, was less than 10⁻⁶ of the input amount, which for our purposes was negligible. The nucleocapsid preparations thus obtained were practically free of neuraminidase and haemagglutinin as determined by gel electrophoresis, but contained a considerable amount of matrix (M) protein and a few minor components (one of which may correspond to viral transcriptase), in addition to NP-protein.

A comparison of circular dichroism (CD) spectra of the native and the phosphorylated nucleocapsid is given in Fig. 1a. Phosphorylation caused a significant reduction in negative ellipticity in the range 200–250 nm, suggesting the reduction of secondary structure content in the protein moiety of nucleocapsid. This conformational change seemed reversible. Thus, the treatment of phosphorylated nucleocapsid with protein phosphatase¹⁰ could remove 80% of the phosphate which had been introduced by the *in vitro* phosphorylation and the CD spectrum of the dephosphorylated nucleocapsid was indistinguishable from that of native one (Fig. 2b). The phosphorylation-induced conformational changes were also reflected in the increased heat lability of the nucleocapsid (insert Fig. 1).

To determine the effect of protein phosphorylation on the nucleocapsid-directed transcription, transcription had to take place in the absence of phosphate donor for protein kinase⁵ and in the present study this was accomplished by using an ATP analogue, β - γ -imido-ATP or AMPPNP (ref. 11). This analogue can be used, although less efficiently, as a substitute for ATP for the viral transcription, but it will not serve as phosphate donor for protein kinases¹¹.

When the native or phosphorylated nucleocapsid was incubated in the ordinary reaction mixture for transcription

(ATP present), the kinetics of the transcription were similar to each other, indicating that the transcriptional activities of both the preparations were equally preserved during the preceding treatment (Fig. 2). In contrast, when transcriptional activities were assayed in a reaction mixture in which ATP was replaced by AMPPNP and therefore free of phosphate donor for protein kinase, the native nucleocapsid exhibited two-phase kinetics of transcription; the reaction rate was significantly low for the first 3 h and then increased. This was observed consistently in six separate

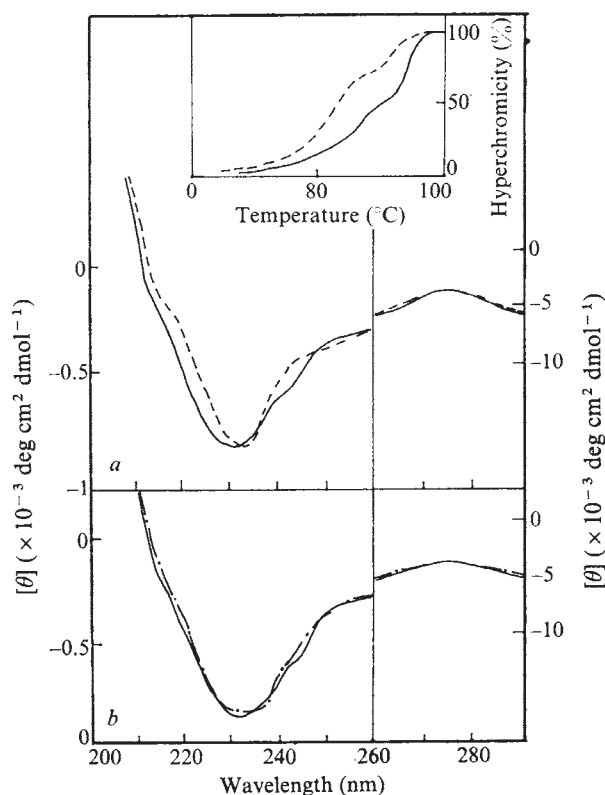


Fig. 1 Comparison of the native and phosphorylated nucleocapsids in CD spectra and in denaturation temperatures. *a*, Purified influenza A₂ virions (3.2 mg protein) were incubated in 3 ml of the mixture for phosphorylation reaction containing 0.1 mM ATP, 8 mM Mg²⁺, 10 mM dithiothreitol (DTT), 0.1% Nonidet-P40 and 10 mM Tris-Cl (pH 8.0) at 32 °C for 30 min. The mixture was cooled in ice and overlayed on a 10% sucrose solution containing 25 mM Tris-Cl (pH 8.0), 1 mM Mg²⁺, and 0.5 mM DTT, and centrifuged at 4 °C and 100,000g for 100 min to pellet the nucleocapsid. The pellet was resuspended and pelleted again through the sucrose solution to remove residual ATP completely. The final nucleocapsid pellet was resuspended in 25 mM Tris-Cl (pH 7.4) to give 0.35 mg protein ml⁻¹ and subjected to the CD spectra determination at 20 °C. The native nucleocapsid was prepared similarly except that ATP was added after the incubation and then removed as above. CD spectra were determined in a Jasco J-20 automatic spectropolarimeter. —, Native nucleocapsid; ---, phosphorylated nucleocapsid. In the range 210–260 nm, the mean ellipticity θ is expressed in deg cm² dmol⁻¹ of amino acid residue (assuming a mean molecular weight of an amino acid as 103) and in the range 260–290 nm deg cm² dmol⁻¹ of nucleotide residue (assuming a mean molecular weight of a nucleotide as 330). *b*, The phosphorylated nucleocapsid described in *a* (2.0 mg protein) was incubated in 5 ml of a mixture containing 10 mM sodium acetate, pH 5.8, 5 mM L-ascorbic acid and 125 μ g protein of partially purified protein phosphatase from bovine spleen¹⁰ at 37 °C for 1 h. The nucleocapsid was then pelleted by sedimentation through the sucrose solution as above and resuspended in 25 mM Tris-Cl (pH 7.4) to give 0.35 mg protein ml⁻¹ for the CD spectrum determination. —, Native nucleocapsid; ---, dephosphorylated nucleocapsid. Inset, Hyperchromicity determinations at 260 nm during temperature rise at 0.5 °C min⁻¹.

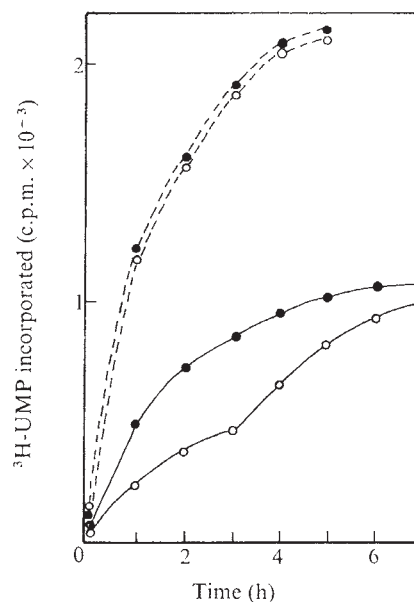


Fig. 2 Transcriptional activities of the native and phosphorylated nucleocapsids in the presence or absence of phosphate donor. The reaction mixture (lacking phosphate donor) contained, in 1.0 ml, 0.1 mM each of AMPPNP, CTP and GTP, 0.2 μ M (20 μ Ci) of ³H-UTP, 8 mM Mg²⁺, 1 mM Mn²⁺, 80 mM NaCl, 5 mM DTT, 10 mM Tris-Cl (pH 8.2) and 2.4 mg protein of the influenza virus nucleocapsid; and was incubated at 33 °C. Aliquots were taken in duplicate at the times indicated and the trichloroacetic acid-insoluble radioactivities were determined in a scintillation spectrometer. In another reaction mixture (containing phosphate donor), AMPPNP was replaced by 0.1 mM of ATP. ●, Phosphorylated nucleocapsid; ○, native nucleocapsid; —, with AMPPNP as substrate; ---, with ATP as substrate.

experiments with different preparations of native nucleocapsid, but such reaction lag was not seen with the several preparations of phosphorylated nucleocapsid. We interpret the results as indicating that the phosphorylation of NP-protein leads to the stimulation of viral transcription. It is conceivable that the conformational change induced by the phosphorylation is involved in the stimulation mechanism. Such stimulation by the previous phosphorylation of nucleocapsid was undetectable in the ordinary transcription system containing ATP, presumably because the phosphate-accepting sites of NP-protein were rapidly saturated in the beginning of transcription reaction⁶.

To examine whether the late recovery of transcription with the native nucleocapsid in the absence of ATP resulted from very slow phosphorylation of the nucleocapsid using CTP, UPT or GTP present, the CD spectra of native nucleocapsid were measured at various times during the transcription. The CD spectrum change below 250 nm which was characteristic to the phosphorylation of nucleocapsid (Fig. 1) was not seen; however, instead, a marked ellipticity change was observed in the range 270–280 nm at about 3 h of incubation (spectra not shown). The ellipticity change at 273 nm as a function of incubation time was plotted (Fig. 3) and the results indicated that the nucleocapsid underwent conformational rearrangement (possibly the changes in RNA-protein interaction) which was distinct from the conformational change caused by phosphorylation of NP-protein, and that the time of rearrangement coincided with the start of late stimulation of transcription. Since this rearrangement also occurred in the absence of nucleotidetriphosphates (Fig. 3c), it was neither the consequence of transcription nor of phosphorylation. Although it is

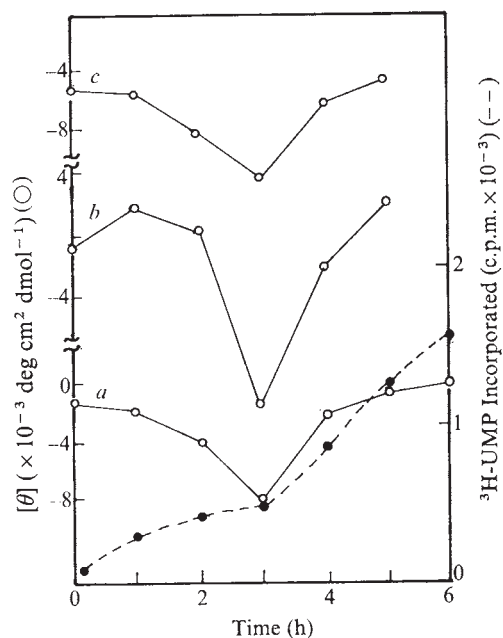


Fig. 3 The ellipticity changes at 273 nm during the transcription with native nucleocapsid. The native nucleocapsid (1.1 mg) was incubated in a CD measuring cell at 33 °C in a final volume of 1.3 ml of the reaction mixture for transcription (lacking phosphate donor) described in Fig. 2. The concentrations of AMPPNP, GTP and CTP were reduced to 0.01 mM so that the CD spectra could be read directly during the incubation. At the times indicated 20 μ l aliquots were withdrawn for the determination of ³H-UTP incorporation into the trichloroacetic acid-insoluble fraction (transcription, — — —), immediately after which the CD spectra were determined and the ellipticities at 273 nm were plotted (a), ———. Each CD measurement took about 10 min and this time was not included in the total incubation time. b, The ellipticity change at 273 nm incubated in the reaction mixture which contained ATP instead of AMPPNP. c, The ellipticity change at 273 nm incubated in the reaction mixture from which all the nucleotidetriphosphates were omitted.

unknown at present whether this conformational rearrangement is an artefact due to the long incubation of isolated nucleocapsid, the result supports the concept that the native influenza virus nucleocapsid must undergo some conformational changes in order to be transcribed efficiently. This effect is not observed, however, in the ordinary transcription reaction using ATP, or in the reaction with pre-phosphorylated nucleocapsid using AMPPNP because the transcription is stimulated more effectively by the phosphorylation of nucleocapsid with the endogenous protein kinase.

TOHRU KAMATA
YASUSHI WATANABE

Department of Molecular Biology,
Tokai University, School of Medicine,
Isehara, 259-11, Japan

Received 24 January; accepted 27 March 1977.

- Lamb, R. A. *J. gen. Virol.* 26, 249-263 (1975).
- Imblum, R. L. & Wagner, R. R. *J. Virol.* 13, 113-124 (1974).
- Moyer, S. A. & Summers, D. F. *J. Virol.* 13, 455-465 (1974).
- Hatanaka, M., Twiddy, E. & Gilden, R. V. *Virology* 47, 536-538 (1972).
- Watanabe, Y., Sakuma, S. & Tanaka, S. *FEBS Lett.* 41, 331-334 (1974).
- Sugiyama, K., Kamata, T., Shimizu, K. & Watanabe, Y. *Jap. J. Microbiol.* 20, 227-232 (1976).
- Bishop, D. H. L., Roy, P., Bean, W. J., Jr & Simpson, R. W. *J. Virol.* 10, 689-697 (1972).
- Caliguiri, L. A. & Compans, R. W. *J. Virol.* 14, 191-197 (1974).
- Rochovansky, O. M. *Virology* 73, 327-338 (1976).
- Rabinowitz, M. in *Methods in Enzymology*, VI (II) (eds Colowick S.P. & Kaplan, N.O.) 218-223 (Academic, New York).
- Yount, R. G., Babcock, D., Ballantyne, Wm. & Ojala, D. *Biochemistry* 10, 2484-2489 (1971).

3' Terminal sequences of 16S rRNA do not explain translational specificity differences between *E. coli* and *B. stearothermophilus* ribosomes

DIRECT Watson-Crick base pairing between the 3' terminus of 16S RNA and messenger RNA seems to contribute to the recognition and binding of protein synthesis initiator regions by *Escherichia coli*^{1,2}. Since bacterial species differ in the pyrimidine-rich sequences at the 3' termini of their 16S RNAs, it has been suggested that this mRNA-rRNA interaction can account for observed differences in translational specificity among prokaryotes³. To test this hypothesis, we have asked whether it explains the choice of sites bound by *Bacillus stearothermophilus* ribosomes in two mRNAs—the R17 and Q β phage genomes. The RNA sequence data presented here lead to two conclusions. First, *B. stearothermophilus* ribosome recognition of two non-initiator Q β regions at high temperature⁴ is indeed correlated with an extensive mRNA-rRNA match. Second, surprisingly, the 3' terminal sequence of 16S rRNA from *B. stearothermophilus* is identical to that of *E. coli*⁵⁻⁷ except that the 3' terminal adenosine of *E. coli* rRNA is replaced by UCUA_{OH} in the thermophile. Thus, rRNA sequences at the 3' end of 16S rRNA cannot explain differences in the capacity of ribosomes from these two species to recognise RNA phage initiator regions at lower temperatures^{4,8,9}.

The translational specificity of ribosomes from *B. stearothermophilus* has previously been studied both at elevated temperatures (60-70 °C), where protein synthesis in this organism is optimal, and at a lower temperature (49 °C) where these ribosomes can be directly compared with those of *E. coli*. At 65 °C *B. stearothermophilus* ribosomes bind efficiently to the A protein initiator region in R17 RNA, while discriminating against the other two phage cistrons^{4,10} (Table 1). In Q β RNA none of the three normal initiation sites is bound by *B. stearothermophilus* ribosomes at high temperature. Instead, two non-AUG- or GUG-containing regions, one of which is recovered as a 35-nucleotide fragment (the typical size of ribosome-protected regions) and the other as a simple purine-rich nonanucleotide, are selected⁴. At a lower temperature (49 °C), where *E. coli* ribosomes strongly bind all three initiator regions in R17 RNA, *B. stearothermophilus* ribosomes retain good binding of the R17 A site and recognise the R17 replicase initiator region only weakly¹¹. With Q β RNA at 49 °C, the coat and replicase initiators are bound, but with much lower efficiency than when *E. coli* ribosomes are used to protect the same RNA¹¹. In addition, the non-initiating Q β sites bound at 65 °C by *B. stearothermophilus* ribosomes are nearly absent at 49 °C (ref. 11 and M. L. Goldberg and J. A. S., unpublished). A reasonable explanation for their disappearance is masking by RNA secondary structure existing at this temperature. Evidence that secondary structure can indeed sequester potential ribosome binding sites comes from observations on the RNA bacteriophage RNAs, where either fragmentation¹²⁻¹⁴ or unfolding with formaldehyde^{15,16} increases ribosome recognition of both authentic and spurious initiation sites in these mRNAs.

We began by asking whether the sites in R17 and Q β RNA which are selected by *B. stearothermophilus* ribosomes at 65 °C are related to each other in having a particularly high degree of complementarity to the 3' terminal sequence of *B. stearothermophilus* 16S RNA. The partial sequence data previously available^{4,17,18} suggested that this might be the case, but resolution of ambiguities remaining in the sequence of the Q β non-initiating sites as well as extension of the *B. stearothermophilus* 16S

Table 1 Sites bound by *B. stearothermophilus* ribosomes

Binding temperature (°C)	Site	Sequence	Theoretical T_m of mRNA-rRNA complex (°C)	References	
				Ribosome binding data	Sequence data
65	R17 A	CCUAGGAGGUUUGACCUAUG	38	4	10
	Q β noninitiator <i>a</i>	CUGAAAGGGGAGAUUACUCG	29	4	This work, 4
	Q β noninitiator <i>b</i>	GGAAAGGAGC	29	4	This work
49	R17 A	CCUAGGAGGUUUGACCUAUG	38	11	10
	R17 replicase	AUGAGGAUUACCCAUG	-7	11	10
	Q β replicase	AACUAAGGAUGAAAUG	-7	11	14, 27
	Q β coat	AAACUUUGGGUCAAUUUGAUGAUG	-25	11	28
	R17 coat	CAACCGGGGUUGAAGCAUG	-46	4, 11	10
Not bound	16S 3' end	HOAUCUUUCCUCCACUAG ₆	—	—	This work, 17, 18

Data were obtained from ribosome-mRNA binding experiments carried out at 65 °C (ref. 4) or at 49 °C (ref. 11). The mRNA sequences represent only the 5' terminal portions (including the AUG initiator triplet) of the bound sites. They are arranged so that the regions complementary to 16S rRNA are aligned. Italics indicate that portion of the *B. stearothermophilus* 16S rRNA sequence which differs from *E. coli*. At each temperature, all sites bound are shown. Since the Q β A site was not recognised by *E. coli* ribosomes tested in parallel at 49 °C, it is presumed that this region was masked at this temperature (as it is at 37 °C (ref. 13)) and unavailable for binding by *B. stearothermophilus* ribosomes (M. L. Goldberg and J.A.S., unpublished). The melting temperatures (T_m) of the mRNA-rRNA complexes were calculated according to the rules for base stacking interactions and for the formation of double helical structures in bimolecular reactions (ref. 26 and R. C. Yuan, personal communication). For this analysis, the complexes were drawn without bulge loops since the thermodynamic parameters for loop structures are less accurately known than are those for structures without loops. The R17 coat site-rRNA complex can be drawn with a bulge loop in an alternative configuration whose calculated T_m is higher than the value shown. The fact that the calculated T_m values fall below the temperatures at which ribosome binding is actually observed indicates that other molecular interactions on the ribosome contribute greatly to the stability of mRNA-ribosome complexes. In the cases where the T_m values of isolated mRNA-colicin fragment complexes have been measured, there is good agreement between the theoretical and experimental values²¹.

terminal sequence was required to reach a definite conclusion. Accordingly, we determined the nucleotide sequences of the two polypurine tracts in the Q β non-initiators and the entire 3' terminal T1 oligonucleotide from *B. stearothermophilus* 16S RNA.

Since only the polypurine tracts from the Q β fragments were required for analysis, we avoided ribosome binding as an isolation procedure and instead took advantage of the large size and unique composition of these oligonucleotides. We isolated them directly from a total pancreatic ribonuclease digest of ³²P-labelled Q β RNA after fractionation in two dimensions (see legend to Fig. 1). The 3' terminal T1 oligonucleotide of *B. stearothermophilus* 16S rRNA, also ³²P-labelled, was isolated by selective adsorption to dihydroxyboryl cellulose¹⁹. Sequences of all three oligonucleotides were deduced primarily by partial digestion with spleen phosphodiesterase, as shown in Fig. 1. Additional data required to establish the sequence of the 16S terminus was obtained by standard techniques²⁰ as detailed in Table 2.

The sequences presented in Table 1 show that the two Q β non-initiator regions and the R17 A site do indeed exhibit extensive complementarity to *B. stearothermophilus* 16S rRNA. The Q β non-initiators (oligonucleotides *a* and *b* in Table 2) can pair with the 3' end of *B. stearothermophilus* 16S RNA to yield a structure which is nearly as stable as the complex formed between the R17 A site and the *B. stearothermophilus* 16S terminus. This latter complex is calculated to be identical in stability to the R17 A site-*E. coli* rRNA duplex, which has recently been isolated and characterised^{21,22}. In contrast, complexes between the *B. stearothermophilus* 16S terminus and other R17 and Q β initiators are theoretically much less stable (Table 1), a property which may explain their inability to be bound by thermophilic ribosomes at high temperatures.

On the other hand, Table 1 also demonstrates that species-related differences in the degree of mRNA-rRNA complementarity cannot explain the mRNA binding preferences of thermophilic ribosomes when *E. coli* and *B. stearothermophilus* ribosomes are compared at 49 °C.

Table 2 Sequence analysis of the 3' terminal T1 fragment from *B. stearothermophilus* 16S RNA

Digestion with pancreatic RNase Products obtained	Digestions used for identification	Molar yields
AU	Alkaline hydrolysis	1
AC	Alkaline hydrolysis	1.0
C	Alkaline hydrolysis	4.8
U	Alkaline hydrolysis	4.6
Digestion with pancreatic RNase after carbodiimide (CMC) blocking		
AUC	Pancreatic RNase after de-blocking, alkaline hydrolysis	
AC	Pancreatic RNase after de-blocking, alkaline hydrolysis	
UC	Pancreatic RNase after de-blocking, alkaline hydrolysis	
C	Pancreatic RNase after de-blocking, alkaline hydrolysis	
U~ ₃ C*	Pancreatic RNase after de-blocking, alkaline hydrolysis	
Digestion with U ₂ RNase		
A	Alkaline hydrolysis	
UCA	Pancreatic RNase $\pm$ CMC, alkaline hydrolysis	
C(C~ ₄ , U~ ₅)A _{OH}	T ₂ RNase: Cp/Up = 0.93	
	Snake venom phosphodiesterase: pC/pA = 3.7	
	pU/pA = 5.2	
	pC/pU = 0.71	
	Pancreatic RNase + CMC: C	
	UC	
	U~ ₃ C*	

*The expected product, UA_{OH}, was not conclusively identified in these digests, presumably because it was obscured by the streaking of U₃C.

- ³ Shine, J. & Dalgarno, L. *Nature* 254, 34–38 (1975).
- ⁴ Steitz, J. A. *J. molec. Biol.* 73, 1–16 (1973).
- ⁵ Noller, H. F. & Herr, W. *Molec. Biol. Rep.* 1, 437–439 (1974).
- ⁶ Ehresmann, C., Stiegler, P. & Ebel, J. P. *FEBS Lett.* 49, 47–48 (1974).
- ⁷ Sprague, K. U. & Steitz, J. A. *Nucleic Acids Res.* 2, 787–798 (1975).
- ⁸ Lodish, H. F. *Nature* 225, 867–870 (1969).
- ⁹ Lodish, H. F. *Nature* 224, 705–707 (1970).
- ¹⁰ Steitz, J. A. *Nature* 224, 957–964 (1969).
- ¹¹ Goldberg, M. L. & Steitz, J. A. *Biochemistry* 13, 2123–2129 (1974).
- ¹² Steitz, J. A. *Proc. natn. Acad. Sci. U.S.A.* 70, 2605–2609 (1973).
- ¹³ Staples, D. H., Hindley, J., Billeter, M. A. & Weissman, C. *Nature new Biol.* 234, 202–204 (1971).
- ¹⁴ Staples, D. H. & Hindley, J. *Nature new Biol.* 234, 211–212 (1971).
- ¹⁵ Lodish, H. F. *J. molec. Biol.* 50, 689–702 (1970).
- ¹⁶ Lodish, H. F. *J. molec. Biol.* 56, 627–632 (1971).
- ¹⁷ Woese, C., Sogin, M., Stahl, D., Lewis, B. J. & Bowen, L. *J. molec. Evol.* 7, 197–213 (1976).
- ¹⁸ Shine, J. & Dalgarno, L. *Eur. J. Biochem.* 57, 221–230 (1975).
- ¹⁹ Rosenberg, M. *Nucleic Acids Res.* 1, 653–671 (1974).
- ²⁰ Barrell, B. G. *Procedures Nucleic Acids Res.* 2, 751–779 (1971).
- ²¹ Steitz, J. A. & Steege, D. A. *J. molec. Biol.* (in the press).
- ²² Held, W. A., Gette, W. R. & Nomura, M. *Biochemistry* 13, 2115–2122 (1974).
- ²³ Steitz, J. A., Wahba, A. J., Laughrea, M. & Moore, P. B. *Nucleic Acids Res.* 4, 1–15 (1977).
- ²⁴ Isono, K. & Isono, S. *Proc. natn. Acad. Sci. U.S.A.* 73, 767–770 (1976).
- ²⁵ Isono, S. & Isono, K. *Eur. J. Biochem.* 56, 15–22 (1975).
- ²⁶ Gralla, J. & Crothers, D. M. *J. molec. Biol.* 73, 497–511 (1973).
- ²⁷ Steitz, J. A. *Nature new Biol.* 236, 71–75 (1972).
- ²⁸ Hindley, J. & Staples, D. H. *Nature* 224, 964–967 (1969).
- ²⁹ Flessel, C. P., Ralph, P. & Rich, A. *Science* 158, 658–660 (1967).
- ³⁰ Griffin, B. E. *FEBS Lett.* 15, 165–168 (1971).

Repeated helical pattern in apolipoprotein-A-I

THE binding of lipids into globules by the serum lipid-binding proteins depends on the unique structures of these proteins. Many have a high α helix content^{1–4} which is enhanced by the binding of lipid, and their amino acid sequences^{5–8} suggest that the helices are often 'amphipathic' with a long hydrophobic face buried in the lipid surface^{9,10} and an exposed polar face which interacts specifically with the charged head groups of phospholipids such as lecithin. Apolipoprotein-A-I (or -Gln-I) is the larger of the two major human high density plasma lipoproteins^{11–13}, with 245 amino acids and a helix content³ of about 70% in the native complex. Physical measurements^{10,14–16} and a study of the sequence^{10,11} suggest that in several of its complexes reconstituted from phospholipids, the protein has a loosely folded structure of between 9 and 13 helices which float in the surface of a disk-shaped bilayer 5.5 nm thick and 11 nm in diameter^{17,18}, rather as logs float on water. With pure lecithin and A-I protein, each complex contains two protein and about 200 lipid molecules¹⁹. The pattern of non-polar groups in A-I protein is unusually regular, even for a helical structure, and this suggested that the sequence may have evolved by internal gene duplication²⁰ in the same way as some other long repetitive structures^{21,22}. Barker and Dayhoff²³, Fitch²⁴ and I independently found a regular 11- or 22-residue repeat pattern in it. Here I report an analysis of the repeats and discuss their structural significance.

To study the repeat pattern, the sequence was compared with itself by matching together all possible segments of a chosen length, using the comparison matrix method²⁵. There is evidently a strong pattern of repeats (Fig. 1) based on a 22-residue unit, which is itself made of two similar 11-residue halves. Initially two sets of calculations were done to explore the pattern; one with a long span of 29 amino acids to look for weak repeats extending over a long range; the other with a span of 11 to look for shorter features. These showed that the strongest repeats involve residues 120–185, where a pattern of 22 amino acids occurs three times over. Next, these three segments were aligned with one another and used as a basis for interpreting the rest of the pattern objectively. To look for the most consistent alignment a family comparison matrix was calculated, which used an average score between any part of the sequence and corresponding parts of the three aligned units (with span 11 and several sets of threshold values).

The results suggest that the protein divides into several regions: three regular repeats of 22 amino acids, residues 120–185; a fairly strong continuation of the pattern (186–240) which stops short of the C-terminal region (241–245); a strong pattern in residues 84–119, apart from one gap near Trp 112; an interrupted fragment of the main pattern (47–78); residues 1–47, which show no significant trace of the repeat, even though

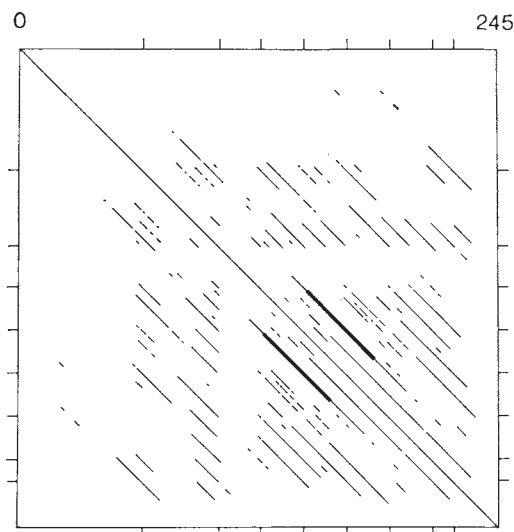


Fig. 1 Comparison matrix for human apolipoprotein-A-I with span 29 and all weights of 1. Points indicate centres of 1,226 off-diagonal spans with scores ≥ 125 . In a random sequence of this composition only 1 in 1,000 or 61 out of 5,970 spans should reach this score. Thick diagonals mark centres of 78 highly significant spans with scores over 146, for each of which the frequency predicted by chance is 1 in 10^6 . Marks along axes show proline residues at positions 65, 103, 124, 146, 168, 212 and 223.

the hydrophobic groups in residues 8–33 have the distribution characteristic of an amphipathic helix.

The best alignment (Fig. 2) is reasonably certain between residues 84 and 221, but the repeat of 11 residues within the basic unit of 22 leads to ambiguities elsewhere, so that several parts of the pattern might be misplaced by 11 residues (for example, near Pro 223). In spite of some uncertainty the observed pattern clearly fits the hypothesis that the protein contains a basic unit of 22 residues, repeated eight times in all.

How significant are the repeats? The answer depends on the null hypothesis, which is assumed. The simplest criterion is to compare the distribution of comparison scores with the expected one for a random sequence of the same composition (for a span length as long as 29, it is necessary to allow for the finite sequence length of 245 residues²⁶). Table 1 shows the distributions for the matrix with span 29 and weights of 1. The number of high scores is very significantly raised in the upper range above 110. In particular, the highest observed score of 162 has a predicted frequency of 10^{-9} and would be reached in less than one random sequence out of 10^4 . The expected top score of 138 is attained in 270 spans instead of two (note that the matrix is symmetric and so each span is counted twice). The odds against such a result in a random sequence are astronomical. Objection might be made that the

Table 1 Apolipoprotein-A-I comparison scores

Score compared with:	Expected frequency	Expected number	Found number
Self			
96	0.26	15,563	16,406
110	0.033	1,973	5,344
119	4.8×10^{-3}	287	2,228
125	1.0×10^{-3}	61	1,226
138	1.9×10^{-5}	1	270
155	2.6×10^{-8}	0	44
162	1.1×10^{-9}	0	2
Myoglobin			
93	0.25	9,555	10,541
105	0.046	1,730	2,386
112	0.011	428	625
119	2.1×10^{-3}	79	90
128	1.6×10^{-4}	6	2

Span 29 and weights of 1. Scoring scheme from ref. 25.

lipoprotein sequence is clearly not random, because of its high content of amphipathic helices, and that the repeats are a natural consequence of this structural feature.

To test this contention similar comparison matrices were run for apolipoprotein-A-I against other proteins with a high helix content: apolipoprotein-C-III (Ala)⁸, human serum albumin²², and sperm whale myoglobin²⁷ (Table 1). In each case there was some enhancement of the number of high scores in the range 110–130, but no really high scores, above 133, were found. Thus the observed repeats in apolipoprotein-A-I are much stronger than can be accounted for merely by a high content of amphipathic helix. It therefore seems most likely that residues 47–240 have evolved by gene duplication from an ancestral unit of 22 amino acids which itself is a doublet of two sets of 11. The tests also suggest that apolipoproteins A-I and C-III do not have a common ancestor, as proposed by Barker and Dayhoff²³.

The characteristic structural features of the pattern (Fig. 3) can be seen in the five most regular repeats (99–207) which are derived from a sequence of the type: (1) Gln-Lys-Leu-Ala-Pro-Leu-Ser-Asp-Glu-Leu-Arg (11); (12) Gln-Arg-Ala-Thr-Gln-His-Leu-Glu-Ala-Leu-Arg (22) (this is not an ancestral sequence but merely a selection of the most common residues in each position). The pattern of hydrophobic residues (period 22) forms a band along one face of the helix, whereas the pattern of polar groups, with a period of 11, consists of a triangle of three negative charges bordered by three positive charges which all lie on the opposite face. The charge distribution is peculiar to this protein and occurs very consistently in the repeats. It seems well adapted to interact with a dipolar lecithin head group in the way proposed by Segrest⁹.

The nature of the repeated motif strongly suggests that the evolutionary repeat unit of 22 residues is also a structural element, which builds an amphipathic α helix with a proline at the amino end, the surface charges of which form a pair of phospholipid binding sites spaced 1.7 nm apart. Notice that three turns of α helix use 10.8 residues, so that a repeat period of 11 or 22 residues is particularly favourable in a helical structure (compare the well known seven-residue repeat in tropomyosin²¹). One notable feature of the sequence as a whole is that an unbroken helix would form a continuous hydrophobic face in long runs including residues 8–33, 44–77 and 86–240. Thus the prolines may not form sharp bends in the lipid complex, but only cause small changes of direction in a series of colinear helices of a total length approaching 30 nm. Such

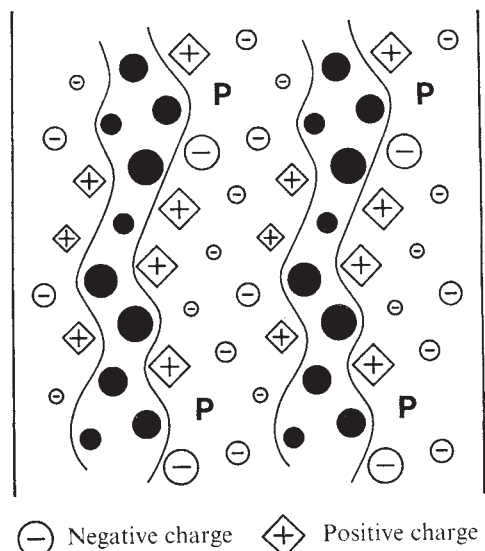


Fig. 3 Helix diagram of 22-residue repeat with 3.6 residues per turn. Sequence reads downwards right to left as if projected on to a sheet of paper wrapped twice round the helix axis and then unrolled. Each residue appears twice to show continuity of pattern clearly. P, Proline; ●, large hydrophobic group. Sizes are proportional to the numbers of each type in residues 99–207.

a structure could run round the circumference of the observed disk-shaped globule. On the other hand, the apoprotein itself without any lipid probably has a compact but loosely folded structure²⁸ which could form by stacking adjacent helices in anti-parallel pairs, rather as in the purple membrane protein²⁹.

Dr Fitch²⁴ has made an interesting phylogenetic analysis of the repeats and I thank him for correspondence.

A. D. McLACHLAN

MRC Laboratory of Molecular Biology,
Hills Road, Cambridge, UK

Received 14 February; accepted 15 April 1977.

- Morrisett, J. D., David, J. S. K., Pownall, H. J. & Gotto, A. M. *Biochemistry* **12**, 1290–1299 (1973).
- Jackson, R. L., Morrisett, J. D., Pownall, H. J. & Gotto, A. M. *J. biol. Chem.* **248**, 5218–5224 (1973).
- Lux, S. E., Hirz, R., Shrager, R. I. & Gotto, A. M. *J. biol. Chem.* **247**, 2598–2606 (1972).
- Jackson, R. L. *et al. J. biol. Chem.* **249**, 5314–5320 (1974).
- Baker, H. N., Gotto, A. M. & Jackson, R. L. *J. biol. Chem.* **250**, 2725–2738 (1975).
- Jackson, R. L. *et al. J. biol. Chem.* **249**, 5308–5313 (1974).
- Brewer, H. B., Lux, S. E., Ronan, R. & John, K. M. *Proc. natn. Acad. Sci. U.S.A.* **69**, 1304–1308 (1972).
- Brewer, H. B., Shulman, R., Herbert, P., Ronan, R. & Wehrly, K. *Adv. exp. Med. Biol.* **26**, 280 (1972).
- Segrest, J. P., Jackson, R. L., Morrisett, J. D. & Gotto, A. M. *FEBS Lett.* **38**, 247–253 (1974).
- Atkinson, D., Smith, H. M., Dickson, J. & Austin, J. P. *Eur. J. Biochem.* **64**, 541–547 (1976).
- Baker, H. N., Delahunty, T., Gotto, A. M. & Jackson, R. L. *Proc. natn. Acad. Sci. U.S.A.* **71**, 3631–3634 (1974).
- Scannu, A. M., Edelstein, C. & Keim, P. in *The Plasma Proteins*, second edit. I (ed. F. W. Putnam) 318–391 (Academic, New York, 1975).
- Morrisett, J. D., Jackson, R. L. & Gotto, A. M. *A. Rev. Biochem.* **44**, 183–207 (1975).
- Finer, E. G., Henry, R., Leslie, R. B. & Robertson, R. N. *Biochem. Biophys. Acta* **380**, 320–337 (1975).
- Forde, T. M., Nichols, A. V., Gong, E. L., Lux, S. E. & Levy, R. I. *Biochem. Biophys. Acta* **248**, 381–386 (1971).
- Scannu, A. M. *Biochem. Biophys. Acta* **265**, 471–508 (1972).
- Muller, K., Laggner, P., Kratky, O., Kostner, G., Holasek, A. & Glatzer, O. *FEBS Lett.* **40**, 213–218 (1974).
- Andrews, A. L. *et al. Eur. J. Biochem.* **64**, 549–563 (1976).
- Hauser, H., Henry, R., Leslie, R. B. & Stubbs, J. M. *Eur. J. Biochem.* **48**, 583–594 (1974).
- Ohno, S. *Evolution by Gene Duplication* (London, Allen and Unwin, London, 1970).
- McLachlan, A. D., Stewart, M. & Smillie, L. B. *J. molec. Biol.* **98**, 281–291 (1975).
- Brown, J. R. *Fedn Proc.* **35**, 2141–2144 (1976).
- Barker, W. C., & Dayhoff, M. O. in *Atlas of Protein Sequence and Structure* **5**, suppl. 2, 253–254 (National Biomedical Research Foundation, Washington DC, 1976).
- Fitch, W. M. in *Proceedings of La Table Ronde on Biomolecular Evolution* (ed. J. Mathieu) (Roussel, Uclaf, Paris, in the press).
- McLachlan, A. D. *J. molec. Biol.* **61**, 409–421 (1971).
- McLachlan, A. D. *J. molec. Biol.* **107**, 159–174 (1976).
- Perutz, M. F., Kendrew, J. C. & Watson, H. C. *J. molec. Biol.* **13**, 669–678 (1965).
- Tall, A. R., Shipley, G. G. & Small, D. M. *J. biol. Chem.* **251**, 3749–3755 (1976).
- Henderson, R. & Unwin, P. N. T. *Nature* **257**, 28–32 (1975).

Fig. 2 Amino acid sequence (one-letter code) aligned to show repeat patterns of 22 and 11 residues. Circles pick out large non-polar groups. Note three strong repeats between 120–185, a gap after Trp 112, breaks at 79–83 and 46, and absence of regular pattern in residues 1–46 and 241–245.

1	(D E P P Q S P (W) D R (V) K D (L) A	15
16	T (V) (V) (V) D (V) L K D S G R D (V) (V) S Q (F) Q G S A	37
38	(L G K Q (L) N (L) K (L))	46
47	(L (W) D D (V) T S T (F) S K (L) R	59
60	Q E (L) G P (V) T E E (W) F N D (L) Q E K (L) N	78
79	(L E K E T)	83
84	G E (L) R Q E (M) S K D (L) E E (V) K	98
99	A K (V) Q P (Y) L D D (F) Q K K (W) - Q E (M) E (L) Y R	119
120	Q K (V) E P (L) R A E (L) Q E G A R Q K (L) H E (L) Q	141
142	E K (L) S P (L) G E E (M) R D R A R A H (V) D A (L) R	163
164	T H (L) A P (Y) S D E (L) R Q R (L) A A R (L) E A (L) K	185
186	E (N G A G R) (L) A E (Y) H A K A T E H (L) S T (L) S	207
208	E K A K P A (L) E D (L) R Q G (L) L P V (L) E S (F) K	229
230	(V S (F) L S A (L) E E (Y) T	240
241	(K (L) N T Q)	245

matters arising

Substitution of guanine for a specific base in tRNA by extracts of Ehrlich ascites tumour cells

We previously reported the partial purification of an enzyme from Ehrlich ascites tumour that catalysed the incorporation of GMP from GTP into specific tRNA molecules¹. The active primer tRNAs for this reaction were found from *Escherichia coli*, Ehrlich ascites tumour, sarcoma 180, and tissues of mice infected with Friend virus, but not from tissues of normal mice. Among the ten purified tRNAs of *E. coli*, only tRNA_{II}^{Leu}, tRNA_{III}^{Ser}, tRNA_I^{Tyr} and tRNA_{II}^{Tyr} were active as primers. These results suggested that, in mouse, primer RNAs are specific to tumours or tissues infected with tumour viruses. We thought that guanosine triphosphate was the actual substrate, because the reaction was neither inhibited by creatine phosphate and creatine phosphokinase, nor by inorganic phosphate, but inhibited by inorganic pyrophosphate.

More recently, however, we have found that the incorporation of radioisotope from ³H-GTP into an acid insoluble fraction was not due to GTP but due to guanine contamination in the reaction mixture. It was shown by thin-layer chromatography on an Avicel SF cellulose plate that about 6% of radioactivity was accounted by ³H-guanine. We have found that α-³²P-GTP was not incorporated in this reaction, and ¹⁴C-GTP was not incorporated. It is very likely that ³H-labelled GTP apparently decomposed to yield ³H-guanine by self-decomposition due to radiation². In fact, the enzymatic incorporation of guanine showed an absolute requirement for acceptor tRNA (Table 1), but no energy source, for example ATP, was required.

Purified *E. coli* tRNAs were tested as acceptors for guanylation by the enzyme (Table 1). tRNA_{II}^{Tyr} and tRNA_{Asp} were very active. Slightly active tRNAs, tRNA_{Asn}, tRNA_{His}, tRNA_{III}^{Ser}, tRNA_{II}^{Leu} and tRNA_{II}^{Val}, were determined with the activities of charging of the specific amino acid. tRNA_{Asn} and tRNA_{His} were not contaminated with tRNA_{Asp} or tRNA_{Tyr}, but tRNA_{III}^{Ser}, tRNA_{II}^{Leu}, and tRNA_{II}^{Val} were found to be appreciably contaminated with tRNA_{Asp} or tRNA_{Tyr}. Thus probably only tRNA_{Tyr}, tRNA_{Asp}, tRNA_{Asn}, and tRNA_{His} act as acceptors. It can be concluded that the primer activity of

tRNA_{III}^{Ser} and tRNA_{II}^{Leu} in the preceding paper¹ is due to such contaminating tRNAs. Four tRNAs that were active in guanylation contained in common the modified base Q in the first position of anticodon^{3,4}.

Table 1. Incorporation of ¹⁴C-guanine into amino acid-specific *E. coli* tRNAs

<i>E. coli</i> tRNAs (A ₂₆₀)	¹⁴ C-Gua incorporated (pmol)
tRNA _{II} ^{Tyr}	0.006
	0.05
tRNA _{Asp}	0.05
	0.5
tRNA _{Asn}	0.05
	0.5
tRNA _{His}	0.05
	0.5
tRNA _{III} ^{Ser}	0.05
	0.5
tRNA _{II} ^{Leu}	0.05
tRNA _{II} ^{Val}	0.5
tRNA _{Pro}	0.5
tRNA _{Glu}	0.5
tRNA _{Met}	0.5
-RNA	0

The enzyme was partially purified by the same procedure described by Itoh *et al.*¹. The standard reaction mixture contained in a total volume of 0.25 ml, 22 μmol of Tris-HCl, pH 8.0, 3.2 μmol of MgCl₂, 0.02 μmol of 2-mercaptoethanol, 10 nCi of ¹⁴C-guanine (55 mCi mmol⁻¹), 30 μg of enzyme protein and each of the indicated tRNAs were added. After incubation at 36 °C for 20 min, the cold 5% trichloroacetic acid-insoluble materials were collected on membrane filters, and the radioactivity of the dried filters was counted by liquid scintillation counter. *E. coli* tRNAs were supplied by Dr S. Nishimura (National Cancer Research Institute).

After tRNA_{II}^{Tyr} labelled with ¹⁴C-guanine by the enzymatic reaction was isolated and treated with RNase T₂, it was fractionated by two dimensional cellulose thin layer chromatography. The radioactive product obtained by the treatment of ¹⁴C-guanine incorporated tRNA_{II}^{Tyr} with RNase T₁ was fractionated by two-dimensional PEI (polyethyleneimine)-cellulose chromatography. The results obtained from these chromatograms and acceptor activities of tRNAs suggested that the enzyme catalysed the reaction to exchange the Q base for guanine at the first position of anticodon.

Farkas and his co-workers previously reported guanylation of tRNA in rabbit reticulocytes^{6,7}. They showed that the guanylated tRNA of rabbit reticulocytes were tRNA_{His} and tRNA_{Asn} (ref. 8). Okada *et al.* recently studied this guanylation reaction in order to identify the exact

position of guanylation and the guanylated species of tRNA. They showed that the Q base in the first position of the anticodon of *E. coli* tRNAs (tRNA_{Tyr}, tRNA_{Asp}, tRNA_{Asn}, tRNA_{His}) was replaced by guanine by the guanylation enzyme from rabbit reticulocytes⁹. It was found that tRNAs isolated from Ehrlich ascites tumour and AH7974 rat ascites hepatoma were active as acceptor for guanylation with the enzyme from Ehrlich ascites tumour, but normal mouse and rat liver tRNAs were not active (ref. 1 and unpublished data). Kasai *et al.* reported that the Q* (a derivative of Q) content of liver tRNA of AH7974 rat ascites hepatoma was twofold greater than that of normal rat¹⁰. One of the major factor of a difference of guanylation activity among these tRNAs is probably the modification of nucleoside such as Q and Q*. Furthermore, a specific structure or sequence common to these tRNAs may be recognised by the enzyme. It will be interesting to examine the structure of tRNAs from tumour cells concerning the guanylation activity.

This work was supported in part by grants from the Ministry of Education, Science and Culture, of Japan, the Waksman Foundation of Japan Inc., the Mitsubishi Foundation, and Keio University School of Medicine Research-aid Fund. We thank Drs S. Nishimura and F. Harada of National Cancer Center Research Institute for help in product analysis and valuable suggestions.

YUKO H. ITOH
TATEO ITOH
ICHIRO HARUNA
ITARU WATANABE

Department of Molecular Biology,
School of Medicine, Keio University,
35 Shinanomachi, Shinjuku-ku,
Tokyo, Japan

Received 22 March; accepted 29 March 1977.

- Itoh, T., Haruna, I. & Watanabe, I. *Nature* **257**, 327-329 (1975).
- E. A. Evans in *Self-decomposition of Radiochemicals*, Review 16, 44-58 (The Radiochemical Centre Amersham, England, 1976).
- Harada, F. & Nishimura, S. *Biochemistry* **11**, 301-308 (1972).
- White, B., Tener, G. M., Holden, J. & Suzuki, D. T. *J. molec. Biol.* **74**, 635-651 (1973).
- Barrell, B. G. in *Procedures in Nucleic Acid Research* (eds Cantoni, G. C. & Davies, D. R.) **2**, 751-779 (Harper and Row, New York, 1970).
- Farkas, W. R., Hankins, W. D. & Singh, R. *Biochim. biophys. Acta* **204**, 94-105 (1973).
- Farkas, W. R. & Singh, R. *J. biol. Chem.* **248**, 7780-7785 (1973).
- Farkas, W. R. & Chernoff, D. *Nucleic Acids Res.* **3**, 2521-2529 (1976).
- Okada, N., Harada, F. & Nishimura, S. *Nucleic Acids Res.* **3**, 2593-2603 (1976).
- Kasai, H., Kuchino, Y., Nihei, K. & Nishimura, S. *Nucleic Acids Res.* **2**, 1931-1939 (1975).

Amino acids in fossil woods

THE significance of the amino acid racemisation date for fossil wood from the Kalambo Falls Prehistoric Site, Zambia, recently reported by Lee, Bada and Peterson¹ requires clarification. The specimen used for ¹⁴C calibration (UCLA-1857), referred to throughout as 'Sangoan wood', is noted on Table 1 of ref. 1 as being 'Kalambo Falls, Site A, River Face (1959) Pit 3, No. 49 (9 ft 9 inch)'.

Because of apparent stratigraphic correlations when this specimen was excavated, it was entered in the field catalogue as being 'Sangoan' in context; this was the notation which was on the sample when it was supplied for racemisation dating. The stratigraphy of the Site A River Face was re-examined in 1963, however, and the section drawing², and field notes, show that this specimen came from a bed in the uppermost White Sands and Dark Clays, from which aggregates including diagnostic Acheulian artefacts were recovered nearby. (The 9 ft 9 inch refers to a field datum; the depth below the published trench datum is 13 ft 9 inch).

That the analyses indicate that the Site A specimen is younger than that from Site B is in agreement with the postulated stratigraphic relationships^{3,4} and palynological evidence⁵. Older ¹⁴C dates have been obtained on wood in equivalent, Site A contexts: >49,000 (GrN 3211); >52,000 (GrN 4228); 61,700±1,300 (GrN 4896)=>60,000 (ref. 5). These would give an older racemisation date for the Site B wood.

Thus, both the analysed specimens from Kalambo Falls have Acheulian cultural associations, and the date of >110,000 for Site B has little bearing on the 'Acheulian-Sangoan transition.' It refers to the Moola Phase of the Bwalya Industry, not to the youngest Acheulian⁶. It is vital that specimens within the ¹⁴C dating range be obtained for calibration, in order to derive finite dates for both of these older woods.

M. R. KLEINDIENST

Scarborough College,
University of Toronto,
West Hill, Ontario,
Canada M1C 1A4

J. D. CLARK

Department of Anthropology,
University of California,
Berkeley, California 94720

C. LEE

Woods Hole Oceanographic
Institution,
Woods Hole, Massachusetts 02543

J. L. BADA

Scripps Institution of Oceanography
and Institute of Marine Resources,
University of California,
San Diego, La Jolla, California 92037

- ¹ Lee, C., Bada, J. L. & Peterson, E. *Nature* 259, 183-186 (1976).
- ² Kleindienst, M. R. in *Kalambo Falls Prehistoric Site 1*, (by Clark, J. D.) 140 (Cambridge University Press, Cambridge, 1969).
- ³ Kleindienst, M. R. in *Kalambo Falls Prehistoric Site 1* (by Clark, J. D.) 151 (Cambridge University Press, Cambridge, 1969).
- ⁴ Clark, J. D. *Kalambo Falls Prehistoric Site 1*, 180 (Cambridge University Press, Cambridge, 1969).
- ⁵ Clark, J. D. *Kalambo Falls Prehistoric Site 1*, Appendix I (Cambridge University Press, Cambridge, 1969).
- ⁶ Clark, J. D. *Kalambo Falls Prehistoric Site 2*, 79 (Cambridge University Press, Cambridge, 1974).

Ductile shear zones and basal aureoles in ophiolite complexes

ATKINSON¹ has recently attributed ductile shear zones in the Bay of Islands ophiolites of Western Newfoundland to the 'transportation and emplacement of oceanic crust and mantle on to a continental margin'. This may be so for some such zones, although the well-exposed basal thrust of the ophiolite-bearing White Hills slice in northern Newfoundland is not associated with either the ductile style of deformation or the 'low greenschist grade' assemblages that Atkinson describes. Moreover, in the Bay of Islands complex there are well defined ductile shears in both the interbanded transition zone between the harzburgites and the gabbros and in the lower parts of the gabbros themselves, that seem to be related to processes near accreting plate margins^{2,3}.

These are best seen in the Lewis Hills, where a wide variety of dunites, harzburgites and gabbros are intensely mylonitised (though rarely to greenschist facies) in zones from several metres wide down to a few centimetres. These zones, many of which are axial-planar to tight folds, are cut by much less deformed dykes and sheets of hornblende gabbro, which seem to have been derived from a tholeiitic parent. Other clear evidence of the overlapping in time of igneous and deformation events is provided by the presence of rotated inclusions of foliated gabbro in relatively massive wehrlite.

It seems then that the ophiolites were undergoing deformation in narrow zones of high strain before they were carried with the spreading ocean floor far from their place of origin at an oceanic ridge or possibly at a transform. It is unlikely however, that the basal aureoles formed in the same environment by the 'lateral flowage of oceanic crust and upper mantle' as Atkinson speculates. Most of the protoliths for these aureoles were distal continental

margin sediments and associated volcanics^{4,5} which were overridden by the ophiolites during their advance onto the continental margin.

A. R. BERGER

R. A. JAMIESON

Department of Geology,
Memorial University,
St John's, Newfoundland

Received 1 February; accepted 29 March 1977.

- ¹ Atkinson, S. J. *Nature* 264, 164-165 (1976).
- ² Berger, A. R. *Trans. Am. Geophys. Union* 55, 558 (1974).
- ³ Berger, A. R., Dewey, J. F. & Karson, J. *Geol. Soc. Am. Anstr.* 7, 25-26 (1975).
- ⁴ Williams, H. & Smyth, W. R. *Am. J. Sci.* 273, 594-621 (1973).
- ⁵ Jamieson, R. A. *Can. J. Earth Sci.* (in the press).

ATKINSON REPLIES—Berger and Jamieson's comments on 'ductile shear zones' in the Bay of Islands ophiolites refer to high-grade structures and assemblages related, in some way, to the formation of Late Cambrian lithosphere at an ocean ridge and to its subsequent lateral flowage during sea floor spreading. I am most familiar with these previously documented structures and agree with this interpretation of their origin.

Ocean floor spreading, however, does not emplace ophiolite sheets on to continental margins. My letter to *Nature*¹ dealt specifically with younger, lower-grade minor structures formed during a late Arenig-Llanvirnian period of ocean basin closure. Crustal shortening was accommodated by telescoping the North American continental margin along major thrust faults. These were singularly responsible for the transportation and emplacement of the Bay of Islands ophiolites on to the continental margin. Field observations of the faults show that they are sharp, clear-cutting surfaces—certainly in no way 'ductile' zones themselves. My reference to ductile deformation zones (DDZ) is in regard to minor structures found within the body of the Bay of Islands ophiolite sheet. These DDZ cut across and retrograde the gabbros, harzburgites, dunites, and 'aureole' rocks, as well as all those structures mentioned by Berger and Jamieson (including the mylonites of higher-grade DDZ).

Clearly, the observations of Berger and Jamieson and previous authors on relatively high-grade DDZ related to ridge and spreading processes are not being challenged here. The observations I have made concern a distinctly later event—one that was responsible for ophiolite transportation onto the continental margin some 40 Myr later.

S. J. ATKINSON

Geology Department,
Saint Mary's University,
Halifax Nova Scotia, Canada

¹ Atkinson, S. J. *Nature* 264, 164-165 (1976).

reviews

Dispelling fears over pesticides

F. Moriarty

Pesticides and Human Welfare. Edited by D. L. Gunn and J. G. R. Stevens. Pp. 278. (Oxford University: Oxford and London, 1977.) Hardback £5; paperback £1.75.

THERE has been a great deal written and said about the alleged disadvantages and dangers of pesticides. This book has been produced in response to this publicity, and is sponsored by seven chemical companies from western Europe, including ICI and Shell from England. The book is intended for laymen and non-specialists, and so discusses, in a lengthy appendix, the meaning of some common technical terms, types of pesticides, their formulation and biological activity. The main text contains three parts, with a total of twenty chapters, each intended to be scientifically accurate, intelligible and authoritative.

There are four chapters in part one. The first three, on the world's food supply (W. R. Furtick), population growth (R. H. Gray), and vector-borne diseases (G. Davidson), introduce the problems that pesticides can help to solve. They combine to illustrate vividly the differences between the industrialised and the developing countries of the world. Data are provided for past and expected future increases in population and food production. It is estimated, for example, that pests consume about 30% of the potential food production in the developing countries, but they use only 10% of the world's pesticides, mainly for public health and exported crops. The developing countries have higher rates of population increase, and, just to maintain present standards, their food production will probably need to almost double during the next twenty-five years. The important point is made several times that methods of food production, medical services and birth rates cannot be considered in isolation, but are all components of a society's culture. Thus, the effective use of pesticides in food production implies a relatively sophisticated agricultural industry. The fourth chapter, by D. J. Ansell, explains some of the financial difficulties in increasing food production in the developing countries.

The second part, with ten chapters, provides examples of how pesticides improve crop yields, animal health and

food storage. The criterion by which pesticides are judged is their cost against the value of the increment in yield. G. Schumann suggests, particularly from German experience, that farmers get a return of between 1:3 and 1:9 for pesticides, going in exceptional cases as high as 1:100. Several other chapters comment, for specific crops, on the lack of precise figures: the introduction to the chapter on tropical cash crops discusses some of the reasons for this, and concludes that in general when pesticides are used their benefits are so obvious that detailed data are unnecessary. Such calculations, when possible, are based of course on a micro-economic analysis. D. E. Jacobs indicates that realistic cost-benefit analyses for an entire community need computer-aided models. J. P. Hudson touches on another aspect of the same problem when he explains that for commercial orchards, once the current standard spraying regimes have been used, it would be disastrous to stop spraying.

The final part, in six chapters, discusses complications of, and alternatives to, pesticides. It is clear that pest resistance to pesticides (J. R. Busvine) is a continuing major worry. The late J. M. Barnes presents a reassuring account of the hazards to people; the major risk comes from human carelessness, not from unexpected toxicity. I am not completely convinced that animals will always be adequate indicators of possible chronic toxic effects in man (p188), but that is a minor quibble. K. Mellanby discusses the "balance of nature" and makes the point that one can never prove that all hazards from pesticides are known. Nevertheless, he sees little cause for

alarm on this score, although I doubt whether we have too much evidence either way for some of the developing countries. D. P. Jones discusses the topical issue of energy costs and gains from pesticides. R. F. Glasser describes some of the controls imposed by governments on the development and use of pesticides, and suggests, as do several other authors, that the increasing costs of research are reducing the amount of research for new pesticides. It would have been interesting to have some comments on the fact that it is becoming more difficult to discover new potential pesticides at the primary screening stage. The final chapter, by D. L. Gunn, discusses alternatives to pesticides and concludes that pesticides will remain of major importance for the foreseeable future.

This book succeeds in its aims. It is clearly written, the few minor misprints causing no problems except for conventional (*sic*) on p212; most of the chapters read convincingly; and the book should inform and stimulate its intended audience. There are some omissions; for example, there are few if any data on quantities of pesticides manufactured and used, and few references to forestry or to herbicides in waterways. The major unanswered query was, where are we going? Clearly, in the short term, we will continue to need and use pesticides. In the long term, if pesticides merely enable us to keep one jump ahead of population increase, for how long will we be able to maintain that state of affairs? □

F. Moriarty is a Principal Scientific Officer at Monks Wood Experimental Station, Institute of Terrestrial Ecology, Huntingdon, UK.

Dynamics of atomic liquids

Atomic Dynamics in Liquids. By N. H. March and M. P. Tosi. Pp. 330. (Macmillan: London, 1976.) £25.

IT is difficult to decide for whom this book is intended. The level of discussion is high and the range covered is very wide. As may be expected the longest chapter is on charged fluids (plasmas, liquid metals and molten salts), a field to which the authors have

made important contributions. Apart from the conventional general discussion of the structure and dynamics of pure atomic liquids and binary liquid mixtures there are short specialised chapters on the helium liquids, the liquid surface and on critical phenomena. Few topics are discussed or explained in any detail, and a considerable expertise in theoretical physics is a prerequisite. Consequently this book cannot be recommended to the beginner. On the other hand it will not be very exciting for the expert, except as a reference book.

The material is not well organised and the

text does not read smoothly—quite apart from many errors of English and poor proof-reading. There is excessive direct quotation of formulae from specialised papers with inadequate indication of the physical assumptions involved. It is clearly difficult to decide in what detail to deal with this wide range of topics in a small book, but the authors have not found a happy solution. Many parts of the book, and its general arrangement, give the impression of very condensed notes for lecture courses. The lecture courses themselves were prob-

bably very good but they are not given here.

In view of the well established reputation of the authors and the significant contributions they have made to the theory of liquids it is to be regretted that they have not produced a book which does them justice.

This will be a useful reference work for experts and it is perhaps in anticipation of this that the price has been set very high.

J. G. Powles

J. G. Powles is Professor of Physics at the University of Kent, Canterbury, UK.

Remote sensing

Introduction to Environmental Remote Sensing. By E. C. Barrett and L. F. Curtis. Pp. ix + 336. (Chapman and Hall: London; Halsted/Wiley: New York, 1976.) £5.95.

THE authors' aim "has been to meet the needs of many students, administrators and scientists for an account of remote sensing which is essentially basic, explanatory and broad in its coverage", illustrating applications as well as techniques. There is certainly a need for such a book to be read, particularly by the administrators and scientists who control in various ways the support (or lack of support) and encouragement for the development of remote sensing and its applications. Does this book satisfy its declared aims?

A range of publications is now available on the subject of remote sensing, from popular accounts, through textbooks and books of collected papers to the *Manual of Remote Sensing* recently published (American Society of Photogrammetry: Falls Church, 1975) in two large volumes. The authors of the book now under review have re-written and edited some of these earlier accounts.

Just over half of the book is concerned with a "selective and illustrative" treatment of applications, covering a range from global climatology to urban studies, and this is perhaps the most useful half of the book for its stated purpose. The atmospheric aspects of the environment receive more attention in this book than in some others, which is not surprising in view of the authorship.

The first half of the book is concerned with the techniques rather than with the applications of remote sensing. The description of the physical basis of remote sensing and the radiation characteristics of natural phenomena reach about the intended level. The chapters on the techniques—that is, the sensors and data processing—are not so satisfactory. Too much previous knowledge is required for this to be included in a basic textbook, and an attempt has

been made to convey too much and too complex information in a small number of words. As one example, in nearly three pages on photographic processing the terms γ , MTF, film stability and granularity, chloride, chlorobromide and bromide papers, and sequential colour printing are introduced among many other topics.

Illustrations and references (including eight pages of colour figures), are both good and plentiful, except that quoted sources of illustrations are frequently not referenced.

It is unfortunate that the text contains enough misprints to be irritating. Occasionally these are factual: for example, the first introduction of ERTS 1 MSS imagery (Plate 3.3, p40) lists the spectral bands wrongly, although it must be said that they are frequently described correctly later in the book.

Overall, this reviewer gives a 'two cheers' reaction—good on applications and illustrations, and reasonably priced in these inflationary days, but barely suitable as a basic textbook on remote sensing techniques.

J. R. Hardy

J. R. Hardy is Senior Lecturer in Geography at the University of Reading, UK.

Human hormones in health and disease

Hormones in Human Blood: Detection and Assay. Edited by H. N. Antoniades. Pp. xvii + 810. (Harvard University: Cambridge, Massachusetts and London, 1976.) £37.10.

THE editor of this multi-author book, Dr H. N. Antoniades, quotes with approval in his Introduction the 1960 statement of Dr G. W. Thorn in his foreword to *Hormones in Human Plasma* that it would be impossible "ever again, to contain within one single volume all information pertaining to this important subject". The dust cover states that *Hormones in Human Blood* "will be an indispensable companion to laboratory workers engaged in clinical and research efforts to measure and understand the role

of human hormones in health and disease". With the book priced at £37.10 this claim merits close examination.

This volume contains 46 chapters contributed by 69 authors covering some 800 pages. The three initial chapters are concerned with plasma proteins and plasma fractionation techniques. The next eight chapters deal with basic aspects of radioimmunoassay methodology. The subsequent and largest section of the book (30 chapters) covers polypeptide hormones and the last section (five chapters), non-polypeptide hormones. A fairly rapid poll of the references quoted suggests that most chapters are completely up-to-date, with 1973 references and 1974 publications of the chapter authors; some go into 1975 and the chapter on growth hormone notably contains 1976 (in press) references which is remarkable as this is the publication year of the book. Does one congratulate the latter authors or identify them as the tardiest contributors?

If it is impossible to be complete in this area how far short of completeness does the book fall? Inevitably it must be in the most rapidly moving new areas rather than in new aspects of existing hormones. For example, of the gastrointestinal hormones only gastrin and glucagon-like substances are considered. This omission is perhaps made more disappointing by the imbalance of the material included. Thus the coverage is considerably affected by the editor's particular interest in protein fractionation and insulin. There are five chapters on insulin immunoassay and five chapters on insulin bioassay. In addition some of this information is also covered in the general chapters on radioimmunoassay methods. One feels, therefore, that a more satisfactory endocrine cover could have been achieved without expanding the book by pruning some of this dated material.

Nevertheless the breadth of coverage provided means that it is impossible for a single reviewer to give an authoritative general opinion of the quality of the contents. Most of the authors are very well established and actively working in their subjects, and therefore their contributions must be very valuable. Inevitably, in looking in detail at the areas with which one is most familiar, one finds defects. For example, must laboratory workers accept the dogmatic statement that the pre-precipitation double antibody method "produces a less sensitive assay than post-precipitation methods", for which the only justification cited is a previous undocumented assertion of the same nature?

One cannot help feeling that the modern laboratory worker in this rapidly moving area will prefer to consult the original literature for most of the methods but will be glad if somebody not too far away has found the money to buy the book.

C. N. Hales

C. N. Hales is Professor of Medical Biochemistry at the Welsh National School of Medicine, Cardiff.

Super Proton Synchrotron project

Europe's Giant Accelerator: The Story of the CERN 400 GeV Super Proton Synchrotron. By Maurice Goldsmith and Edwin Shaw. Pp. 288. (Taylor and Francis: London, 1977.) £13.

IN telling the tale of the Super Proton Synchrotron (SPS) project, Maurice Goldsmith and Edwin Shaw have produced a book that is both fascinating and frustrating. Although it is explicitly stated that the views and comments presented are those of the authors and not the official opinion of the European Organisation for Nuclear Research (CERN), the authors do not often stray from the party line, and the text tends to glorify CERN and praise the success of the SPS. And why not—it is a story to be proud of.

Beginning with a brief and rather high-minded justification for high energy accelerators the authors move straight into the political wranglings that continued for so many years before the SPS project was accepted and funded. A detailed description of the birth-throes is given but its conception is handled more discretely. It will be difficult for an uncommitted reader to become emotionally involved as the political and scientific justification for the SPS is inadequately presented. The reader needs to be emotionally involved to appreciate the detailed description of the implementation of the project which is presented in the rest of the book.

The discussion of the fight for acceptance of the SPS project is fascinating. The brevity of the discussion is frustrating. After rather full-some praise of the CERN convention—the rules by which CERN operates—it comes as a shock that it did not seem to work too well in the case of the SPS. More comment on this would have been welcome, as would be additional discussion of the roles of the various member states during the negotiations. One gets the impression that only a small part of the story has been told—enough to whet the appetite but insufficient to give full satisfaction.

Turning to the building of the machine itself, the authors describe the process in great detail in separate chapters dealing with individual aspects of the problem: surveying, magnets, power and water supplies, radio-frequency acceleration, and so on—topics beloved by engineers and belittled by everyone else, but presented here in an exciting style. Aspects often taken for granted are revealed as complex technical problems and their solution is surprisingly interesting, not

least because of the clear way in which they are presented.

Apt portraits are given of most of the key personnel in the project—a device which adds a great deal of interest to the technological descriptions but, even so, in places far too much technical detail is included and the story becomes difficult to follow. In general, however, there is a good blend of personal interest, technical detail, explanation and discussion of management techniques, which makes a very readable story.

Although the text is fascinating, the layout of the book is frustrating. Pictures are liberally sprinkled throughout; most are of interest but others are fatuous, and the text is fitted into the remaining space after huge margins have been accommodated. If a picture

is encountered, on turning a page one has to search frantically for the text; often there isn't any. Even when there are no pictures the text does not begin until nearly a quarter of the way down the page.

It would have been preferable to expand the scope of the book to put the SPS project into context and explain more fully the motivations and use to which it will be put. This could have been done without increase of the (already high) cost, at the expense of reducing the space taken up by pictures.

This book is one which can usefully be read even though it is produced as if it were a glossy handout for a publicity department. **Stuart Sharrock**

Stuart Sharrock is Physical Sciences Editor of Nature.

Organic reaction mechanisms

Reaction Mechanisms in Organic Chemistry. By Florin Badea. Pp. 701. (Abacus: Tunbridge Wells, Kent, UK, 1977.) £18.85.

THE investigation of organic reaction mechanisms by the application of kinetic measurements originated with Lapworth's work on the halogenation of acetone, at the beginning of the present century. It was not until much later, after 1930, and especially through Ingold's work on substitution reactions, that mechanistic studies became accepted as the cornerstone of the scientific basis of modern organic chemistry. Almost any recent textbook of organic chemistry, even at an elementary level, reflects this view. Several more specialised treatises, including the famous ones by Ingold and Hammett, have given more detailed analyses of the problems of reaction mechanisms in relation to the structure of organic compounds. The work now under review is a recent addition to the literature in the second category. Its author is Professor of Organic Chemistry at the Polytechnical Institute, Bucharest, Romania, and we learn that his research experience included a period in Donald Cram's laboratory at UCLA. The book is well translated (by the author, with help) into readable, though not altogether faultless, English.

The text starts with an introduction to group theory, an account of the Hückel molecular orbital (HMO) method, some rudimentary thermodynamics and statistical mechanics, a statement of the principles of chemical kinetics, and a brief survey of reaction intermediates. These sections are

followed by a detailed treatment of mechanisms of a large number of selected reactions, beginning with aliphatic substitution and ending with cycloadditions. The level of the treatment is intended for postgraduates and advanced undergraduates.

In its 700 crowded pages the book manages to include a vast amount of material and many references. Much of the information is of recent date, and it is not surprising that frequently the aptness of its selection is at least debatable. Clearly, in such an enormous undertaking, there are also bound to be errors of fact or interpretation. Some of these are excusable and could prove a challenge to a good student. The author does not generally attempt unconventional interpretations or to resolve conflicting views. He is not afraid to show his limitations and to leave the reader to develop his own critical faculty by sifting the arguments presented.

Probably the least satisfactory aspect of the book is the absence of any coherent attempt to explain the current methodology of the elucidation of reaction mechanisms. This is not a text from which a student could gain a satisfactory understanding of the application of kinetic methods and become aware of its pitfalls, for example. The treatment of topics such as the role of pre-equilibria, the stationary-state hypothesis, kinetic salt effects, or kinetic isotope effects is inadequate and sometimes seriously in error.

Nevertheless, the book is quite good in parts but, as better introductions to the subject are available in the English language, it is unlikely to be widely adopted.

V. Gold

V. Gold is Professor of Chemistry at King's College, University of London, UK.

Dynamics of fish populations

Fish Population Dynamics. Edited by J. A. Gulland. Pp. xi + 372. (Wiley-Interscience: London and New York, 1977.) £13.50; \$25.

THIS book describes how the dynamics of fish populations can be analysed in terms of the factors affecting their rate of growth, mortality and reproduction, with particular emphasis on the effects of fishing. There are sixteen contributors to the book, including the editor, and many of them have a long-standing international reputation in this field, so much so, that the chapters by Ricker, Gulland, Cushing, Regier, Larkin and Allen, and Chapman are bound to guarantee the book the success it well deserves. It is intended primarily for those already having some interest in fishery science, and for graduates and final-year undergraduates in biology and related subjects. Its form of presentation and style will make it a very 'usable' reference for fishery science undergraduates.

The book has been arranged in three parts. In the first chapter, Ricker describes the historical development of the subject and includes the quantitative methods, of increasing reliability, for describing the direct effects of fishing on stocks of fish and for predicting the catches expected to result from changes in the intensity of fishing or in the sizes of fish caught.

The next section describes some of the particular lines of investigation and methods used in more detail. Because direct observation of fishing in the oceans is difficult and expensive if carried out by special research vessels, scientists have for long depended very much on information from the commercial fisheries; Williams describes the practical problems met in collecting this data. This is a particularly valuable chapter for undergraduates and recent graduates in making them aware of the problems facing the fisheries scientist in obtaining reliable data.

The main lines of analysis as used at present, are reviewed by Gulland, whereas further lines of advance are explored by Rothschild (concerning the concepts of fishing effort and fishing mortality), by Cushing (the problem of the relation between adult stocks and the number of young produced) and by Regier (the position of the fish stock in the whole ecosystem).

The third part of the book looks at how the ideas described earlier have worked out in practice when applied to actual populations. Fish population dynamics grew up in the North Atlantic and North Pacific and much of the work was done on three fish—cod, plaice and the five species of Pacific salmon. The present state of understanding of these species is reviewed by Garrod, Bannister and Larkin, respectively. Population studies and the application of these studies to practical problems, are now worldwide. Murphy looks

at the clupeoids, representatives of which are found in most parts of the world, and Rothschild and Suda look at the larger tuna species, most of which are found in all the warmer oceans. The studies reveal how many of the ideas developed in relations to a few fish in northern temperate areas have been usefully applied to very different areas and to very different fish, but Holden shows how some basic differences in the biology of elasmobranchs require some important changes in approach. Finally, Allen and Chapman examine some aspects of the dynamics of whale stocks and in so doing give us a very useful history of whaling.

For those with little understanding of statistics this is not a book to shy away from, as it is most readable and easily understood. It is also not without its lighter moments, as when Gulland (p70), when referring to the difficulty of making direct observations on fish at sea in mid-winter, states that it "involves a large, seaworthy ship costing hundreds of pounds to run. Most fishery research laboratories have one

or more specially equipped research vessels to carry out this sea work, but their high running costs and the need to justify their even higher capital costs often cause them to hang like gold-plated albatrosses round the necks of the directors of the laboratories concerned."

In addition, the political aspects of fisheries appear from time to time, as when Larkin (p183) refers to the fact that there is no international agreement that excludes participation of all-comers in the harvesting of salmon beyond narrow coastal limits and, as he says, "at present prices for salmon, it seems only a matter of time before someone decides to test the political wind."

This is a relatively expensive book but is one which the budding fisheries scientist should not be without. **Derek Mills**

Derek Mills is Lecturer in Ecology and Fisheries Management in the Department of Forestry and Natural Resources at the University of Edinburgh, UK.

Continental drift

Continents in Motion: The New Earth Debate. By Walter Sullivan. Pp. xiv + 399 + 29 plates. (Macmillan: London and Basingstoke, 1977; McGraw-Hill: New York, 1974.) £6.95.

THIS book is aimed at the general intellectual, non-specialist reader and is undoubtedly the best book on continental drift which has yet appeared for this type of market, although its numerous anecdotes and overview makes it interesting reading even for the specialist. It was originally published in the United States in 1974 and, although it is regrettable that it has not been up-dated, the information it contained at that time was so up-to-date that subsequent data have only extended and modified certain concepts. This new publication is therefore only slightly dated.

It is a large well produced book and contains 29 colour photographs, although some of these could have been dispensed with and further black-and-white diagrams inserted. It is written, as would be expected by the Science Editor of the *New York Times*, in the best journalistic tradition—with an easy style, full of human as well as scientific interest, yet avoiding the slickness and doomwatch aspects which often spoil such books. The drawback for a non-American reader is the emphasis on American findings: most examples are taken from America or American authors, although the British contributions are amply acknowledged. Nevertheless, Arthur Holmes

still only gets a few lines, whereas the Velikovsky farce receives almost a full chapter.

There are hardly any factual errors and most do not really matter for the audience at which it is aimed, although diagrams of the *expected* magnetic anomaly patterns in the western Mediterranean should not be portrayed as the actual pattern, even schematically.

I was more disappointed that Sullivan had not really contributed himself, although this is understandable as he has mainly been a bystander. Throughout, however, there was a sneaking feeling that he has already thought of, but not voiced, the questions which the specialists should be asking themselves, but which have not yet been recognised as the real problems.

The poorest coverage is on mechanisms as the concentration is very much on plumes, with brief consideration of the possibility of shallow convection, but virtually no regard to the probable dominant factor, large scale mantle-wide convection; but this may merely reflect the reviewer's prejudices! Surprisingly, the metal-ores, other than those of the seafloor, receive little attention, although petroleum and particularly geothermal energy resources are covered. But to ask for more is really a compliment to what has been included.

The book can be unreservedly recommended to the general reader, and even specialists would find £6.95 could provide them with numerous insights and amusement, even if they learn few new facts. **D. H. Tarling**

D. H. Tarling is Senior Lecturer in the Department of Geophysics and Planetary Physics at the University of Newcastle upon Tyne, UK.

newly on the market

These descriptions are prepared by the staff of *Nature* on the basis of material provided by manufacturers. The Reader Enquiry Card faces the inside front cover.

Plasma Emission Spectrometer. Tech-
mentation. The Spectraspan IV, a single
element plasma emission spectrometer
can perform quantitative and qualita-
tive analyses of trace concentrations
including refractories and some non-
metals. It has a high sensitivity even
in the presence of complex matrix
solutions with solids contents as high
as 20%. The high intensity excitation
source is a d.c. argon plasma which
operates at low power but produces
temperatures as high as 10,000 K.
An integral microprocessor increases
efficiency and allows routine perform-
ance checks.

Circle No. 42 on Reader Enquiry Card

Radiation dosimeter in SI units. Dosi-
meter. A radiation dosimeter in SI
units, the Model 883 is calibrated
0–129 $\mu\text{Ci kg}^{-1}$ and 0–500 mR.

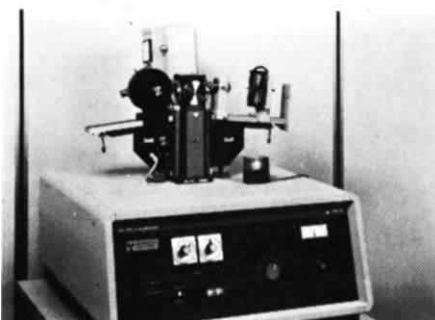
Circle No. 43 on Reader Enquiry Card

Graphite furnace. Perkin-Elmer. The
HGA-2100 graphite furnace, a flame-
less sampling system is for use with
atomic absorption spectrophotometers
has the new gas stop and mini gas flow
system which controls sensitivity
during atomisation. This graphite fur-
nace can determine most metallic
elements with detection limits up to
1,000 times better than those obtained
with conventional burner systems.
Solution volumes as small as a few
tenths of a microlitre can be analysed
as easily as larger samples of up to
100 μl required when measuring extre-
mely low concentrations or non-
homogenous materials. Addition of a
temperature ramp accessory provides
a convenient means of automatically
increasing temperature during all three
phases of operation, dry, char and
atomise. The HGA-2100 can also
analyse many solid samples directly—
materials that have been successfully
analysed without prior dissolution in-
clude plant tissue, polymers, finger
nails and paper products. The deter-
mination of metallic elements in diffi-
cult matrices is also simplified by the
design of the gas flow and sample
introduction systems. Lead can easily
be determined at low levels in 6 N
solution of sulphuric acid.

Circle No. 44 on Reader Enquiry Card

UV-Vis spectrophotometer. Perkin-
Elmer. The dual wavelength, double
beam, UV-Vis Spectrophotometer,
Model 556, is suited for applications in-
volving turbid samples, mixture analysis
and other biochemical and industrial
applications. Two separate diffraction
grating monochromators, with indepen-
dent selection of wavelength, produce
the sample and reference beams which
pass along identical paths through the
sample cell. To record the spectrum of
a turbid sample where no reference
solution exists, one monochromator can
be fixed and the other set to scan.
Alternatively, the two monochromators
can be fixed a few nanometers apart
and both scanned together to record
the first derivative of a spectrum.
Double beam operation is with both
sample and reference beams taken from
one monochromator. Regular differ-
ence spectra can be recorded with a
high performance level.

Circle No. 45 on Reader Enquiry Card



Philips new PW 1720 table-top X-ray
generator equipped with Debye-Scherrer,
Universal flat plate and Guinier cameras.

Table-top generator. Philips. The
PW 1720 top measures 750×700 mm
and with standard Philips tube shield,
will easily accommodate single crystal
goniometers and Debye-Scherrer, Gui-
nier or Universal flat plate cameras. A
vertical goniometer for diffractometry
can also be fitted. The height of the
generator top is 380 mm, enabling it to
be placed on a table and provide an
ideal working height for camera align-
ment. The basic generator is full wave
rectified, with rated power consump-
tion of 2,500 VA max., and has full
tube current stabilisation, continuously
adjustable between 5 and 60 mA. Cur-
rent stability is <0.3% per % mains
voltage variation of $\pm 10\%$, with drift
<0.2% per $^{\circ}\text{C}$ at ambient temperature.

Circle No. 46 on Reader Enquiry Card

Thermal imaging camera. Marconi-
Elliott. The pyro-electric camera has a
special Vidicon camera tube which re-
sponds only to heat radiated from a sub-
ject with no contribution whatever
from light in the visible spectrum. The
camera gives an output which is com-
pletely compatible with standard 625
line TV equipment. This, and its por-
tability, has been demonstrated by its
use as a 'roving' camera. Hitherto
thermal imaging cameras involved
highly complex and expensive forward
looking infrared (FLIR) systems, based
on the use of a large matrix of discrete
heat sensing elements, in conjunction
with a very high speed mechanical
scanning system. Their cost greatly
limited the application of thermal
imaging techniques. The Vidicon
camera, whose construction and opera-
tion is similar in principle to that of a
conventional TV camera, can extend
the technique to a variety of industrial,
medical and even domestic uses. Work-
ing with virtually any TV monitor the
camera can show pictures which bear
a remarkable resemblance to those
taken by cameras using visible light
using only the pattern of thermal
energy produced by the object. Thus
'hot spots' in rotating machinery, in-
cluding for example, vehicle tyres, can
be readily located as can thermal stress
in power cables and soil patterns in
aerial surveys. For medicine a camera
of this kind could have application in
thermography for the diagnosis of
tumours. For surveillance, an intruder
can be detected even in total darkness.
Its use in defence equipment is also
significant since, by not requiring to
irradiate the object observed with in-
frared energy, the presence of the
observer cannot be detected.

Circle No. 47 on Reader Enquiry Card

**Programmable multiple reagent dis-
penser.** Dynatech. The Dynadrop de-
livers up to 12 individual reagents to
a Microtiter plate in quantities from 10
to 200 μl within an overall accuracy of
 $\pm 2\%$. Individual reagents are con-
tained in a rack of test tubes which
in turn is located in a low pressure
reservoir. An integral pump provides
filtered supply air at up to a maximum
of 5 lbs inch^{-2} . The reservoir, connect-
ing tubes and dispense manifold are
all autoclavable.

Circle No. 48 on Reader Enquiry Card

Digital radiometer. Ultra-Violet Products. The J-260 digital radiometer measures UV intensities rapidly and automatically. Digital readout in radiometric units is within a broad dynamic range of $1 \mu\text{W cm}^{-2}$ to 199.9 mW cm^{-2} with accuracy, traceable to NBS, $\pm 1.0\%$ of full scale. High sensitivity for measuring low level irradiance is to the order of ± 1 digit. The radiometer is available with cosine-corrected sensors for measuring at 254, 297 or 365 nm. It is fully portable, uses alkaline or rechargeable NiCd batteries and is supplied complete with choice of sensor, batteries and recharger.

Circle No. 49 on Reader Enquiry Card

Navigational echosounder. Marconi. The Seahorse features possibly the largest chart to be found on any known navigational echosounder, an integral readout, automatic range change facilities and a depth alert. Optional extras are a large size remote digital readout and a large analogue meter-type display. Seahorse provides two depth ranges 0–100 m and 0–1,000 m, and a depth alert system.

Circle No. 50 on Reader Enquiry Card

Portable infrared gas analyser. Wilks Scientific. The Miran 104 portable gas analyser is a light-weight 14.5 m variable path length gas cell which, when allied to the Miran spectrometer, produces a high sensitivity and specificity analyser.

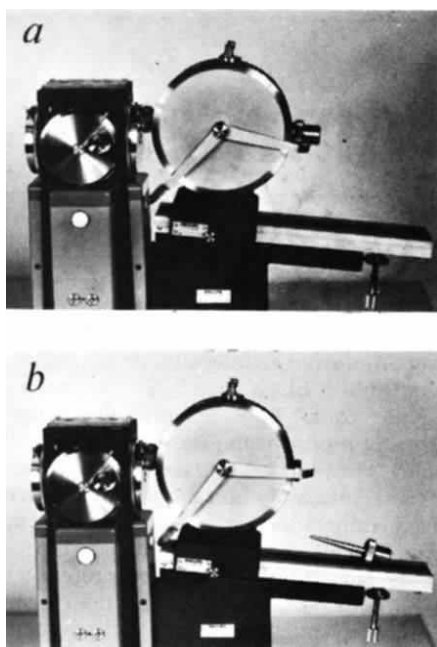
Circle No. 51 on Reader Enquiry Card



Wang PCS II

Deskette computer. Wang. The Wang PCS II Personal Computing System, is the first desktop computer available with minidiskette storage devices. The PCS II has a 1,024-character CRT screen; a typewriter-like keyboard with separate numeric keypad and 32 special function keys for data entry and control; a high-speed processor with two memories: 8 kbytes of user-available memory and 42.5 kbytes reserved for the 2200 operating system and BASIC language interpreter, and either one or two minidiskettes, each of which stores 89,600 bytes of information. The complete PCS II system weighs 29 kg.

Circle No. 52 on Reader Enquiry Card



a, Debye-Scherrer cameras. Camera correctly installed—safety arm activates microswitch to open beam shutter.
b, Part of camera (exit tube) removed—safety arm de-activates microswitch to close beam shutter.

Debye-Scherrer Cameras. Philips. The PW 1024/30 and PW 1026/30 cameras can be used with any Philips tube tower provided with an external safety microswitch, and use the standard diameters of 114.83 mm and 57.54 mm respectively. An important safety feature is a counter-balanced arm which can only activate the safety microswitch—to open the tube tower shutter—when the camera is correctly installed. If the camera, or any part of it, is removed the arm releases the microswitch and the tube tower shutter immediately blanks-off the X-ray beam. The arm also prevents the inadvertent use of an entry collimator in place of the correct exit tube. Exit tubes are now of one-piece construction so that removal of the lead safety glass is impossible.

The camera body and pedestal are formed as a single integrated unit so that alignment relative to the tube tower exit is automatically correct. Accuracy is improved because the collimator and exit tube are always in correct alignment.

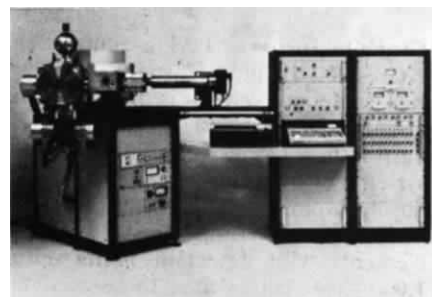
Circle No. 53 on Reader Enquiry Card

Portable noise monitor. Radiatron Instruments. A self-contained portable noise monitor, designed to calculate and print-out community noise levels the Model 614 will print out a wide variety of noise data. The instrument can be equipped to provide a threshold-controller single event printout (such as a flyover) including start and stop time and maximum noise level.

Circle No. 54 on Reader Enquiry Card

Mass spectrometer. Varian MAT. The MAT 260 spectrometer system is intended for automatic precision analysis of isotope ratios in solids (U, Pu, Rb, Sr, Pb and others). A programmable desk top calculator HP 9825 A is an integral component of the mass spectrometer system, optimising the ion source, controlling analysis procedures as well as evaluating and listing results. Twelve masses can be preselected for each measuring cycle, the user having the choice of manual 'one-off' measurements and fully automatic analyses of up to 13 samples mounted in a sample magazine. The exceptional performance of the MAT 260 is due to the high efficiency of a totally new ion source employing two einzel lenses, and the near 100% transmission of the newly developed stigmatic focusing analyser, the resolving power of which corresponds to that of a conventional 46 cm radius system. System sensitivity is better than one ion per 1,000 atoms of uranium. Internal reproducibility is better than 7×10^{-5} . Optimum vacuum conditions are generated by a turbo-molecular pump, two getter ion pumps and a cryo pump. The pumping system is almost maintenance-free and requires no cooling water connection. Devices for sample handling and preparation are included in the system.

Circle No. 55 on Reader Enquiry Card



Varian MAT 260 Spectrometer system

Conductivity meter. Pye Unicam. The PW9505 has a choice of 10 standard measuring ranges from 0 to $3 \mu\text{S cm}^{-1}$ up to 0–100 mS cm^{-1} . A single specially developed plastic conductivity cell PW9514/60 covers all these ranges. The large 0.5% class meter minimises reading errors and gives high accuracy and facilities are provided for conductimetric titrations, reference measurements and ordinary resistance measurements. Reference temperature is normally 20 °C but it can be extended to 25 °C with a minor modification. Manual or automatic temperature compensation is provided. The manual mode covers the range 0–50 °C. The automatic mode, using the Pt100/1 resistance thermometer or reference cell, covers the range 0–100 °C.

Circle No. 56 on Reader Enquiry Card